SERODIAGNOSIS OF DENGUE IN PAEDIATRIC PATIENTS

IMPORTANCE OF NS1 ANTIGEN

Dissertation submitted in partial fulfillment of the
Requirement for the award of the Degree of
M.D. MICROBIOLOGY

BRANCH IV

DEPARTMENT OF MICROBIOLOGY
TIRUNELVELI MEDICAL COLLEGE,
TIRUNELVELI - 620711.

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APRIL 2013.

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This is to certify that the Dissertation **‘‘Serodiagnosis of Dengue in Paediatric patients-Importance of NS1 Antigen”** presented herein by **Dr.C.CHITRA** is an original work done in the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch IV) Microbiology under my guidance and supervision during the academic period of 2010 - 2013.

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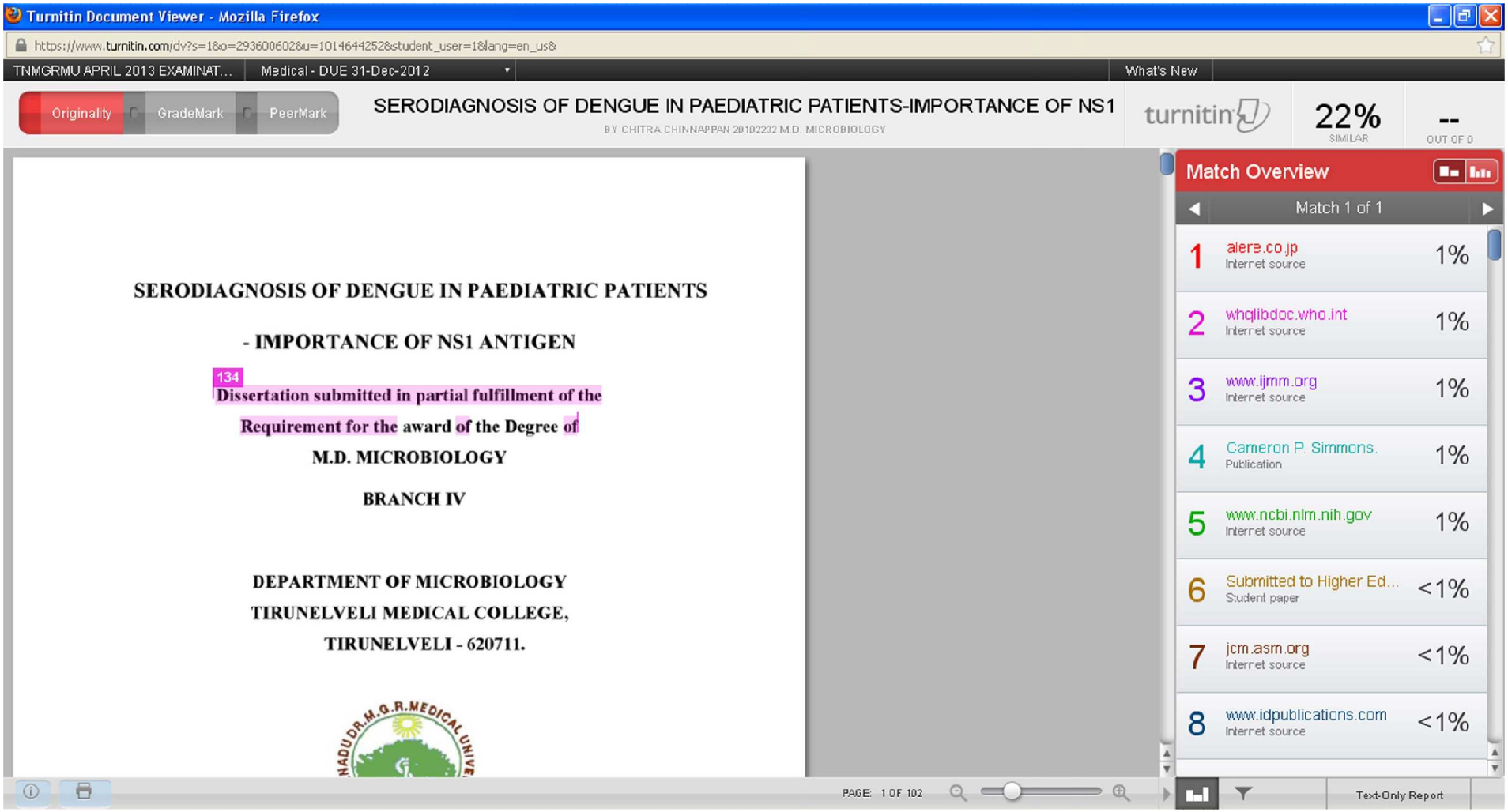
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I solemnly declare that the dissertation titled **"Serodiagnosis of Dengue in Paediatric patients-Importance of NS1 Antigen"** is done by me at Tirunelveli Medical College hospital, Tirunelveli.

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The Ethical Committee of Tirunelveli Medical College and hospital has approved the study,

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ABBREVIATIONS

ELISA	-	Enzyme linked Immuniosorbent assay
DF	-	Dengue fever
DHF	-	Dengue Haemorhagic fever
DSS	-	Dengue Shock syndrome
HAI	-	Haemagglutination inhibition test
HRP	-	Horse radish peroxidase
IgM	-	Immunoglobulin M
IgG	-	Immunoglobulin G
NS	-	Non structural proteins
TMB	-	3,3',5,5' tetra methyl benzidine
OD	-	Opitcal Density
Mab	-	Monoclonal antibody
WHO	-	World Health Organisation

Contents

CONTENTS

1. INTRODUCTION

Dengue is a re-emerging, mosquito-borne viral disease in the world. Dengue fever (DF) and its severe forms like dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) have become major international public health concern.[1] Children are at higher risk and the mortality rate during secondary infection is nearly fifteen times higher than the risk in adults.[2] Since there is no immunoprophylaxis or specific antiviral treatment available, timely and rapid diagnosis plays an important role in patient management and application of control measures to prevent high morbidity and mortality.

1.1. History

Dengue fever was first referred as “water poison” associated with flying insects in a Chinese medical encyclopedia in 992 from the Jin Dynasty (265-420 AD). The word “dengue” is derived from the Swahili word Ka-dinga pepo, meaning “cramp-like seizure”. The name Break bone fever was first applied by Benjamin Rush in 1789. The clinically recognized dengue epidemics occurred almost simultaneously in Asia, Africa, and North America in 17th century and were infrequent until 1940. The name Dengue fever came in to use only after 1828. The Aedes mosquito was confirmed as the transmitting agent in 1906, and the causative agent was identified as virus in 1907. The first report of DHF was from Philippines in 1953.[3]

1.2. Global scenario

Over the last five decades the incidence has raised 30 fold and the disease has expanded itself to new geographical locations and also from urban to rural areas. Now Dengue is endemic in more than 100 countries in the regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. The South-East Asia and Western Pacific regions are the most seriously affected.[1]

About 2.5 billion people, the two fifths of the world's population in tropical and subtropical countries are at risk. An estimated 50-100 million dengue infections occur worldwide and around 5,00,000 affected people require hospitalization every year. The mortality rate of DF is less than one percent, for DHF this is approximately 5%, when patient develops DSS it is as high as 40%. A large proportion (approximately 90%) of them are children aged less than five years, and about 2.5% of those affected die of complications.[1]

1.3. Indian scenario

DF/DHF is endemic in most countries of the South East Asian region and detection of all four serotypes has now rendered these countries hyperendemic. As per WHO 2011 report, India is classified under category A for the following reasons, hyperendemicity with all four serotypes in urban areas and spreading to rural areas, the leading cause of hospitalization and death among children and a major public health concern.

The first proved epidemic of DF in India occurred in Eastern Coast of India in

1963-1964[4].

One of the largest outbreaks in India occurred in Delhi in 1996 which was mainly due to dengue-2 virus. Thereafter, in 2003, another outbreak occurred in Delhi in which dengue-3 was the predominant serotype.[5]

1.4. Dengue in Tamil Nadu

Dengue has been rampant in parts of Tamil Nadu in the past two decades. The prevalence of dengue vector and silent circulation of dengue viruses have been detected in rural and urban Tamil Nadu, which is ever increasing.[6]

1.5. Morphology of dengue virus[3]

The dengue virus belongs to the family *Flaviviridae* and genus *Flavivirus*. They are small (50nm) enveloped viruses and the nucleocapsid exhibits cubic symmetry. Their single stranded RNA genome is 11,644 nucleotides in length and contains three structural protein genes encoding the nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E) and seven non-structural protein (NS) genes **NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5**.(Figure 1)

There are four distinct closely related serotypes of Dengue virus **(DENV-1, DENV-2, DENV-3, and DENV-4).** Distinct genotypes have been identified within each serotype. Currently, three sub-types exist for DENV-1, six for DENV-2, four for DENV-3 and four for DENV-4.[1,3]

1.6. Structural Proteins

1.6.1. C Protein

Nucleocapsid or core (C) protein is the first viral polypeptide synthesized during translation. It protects the viral nucleic acid from inactivation and introduces the viral genome into host cells.

1.6.2. M protein

The membrane associated protein M is derived from the precursor protein prM during virus maturation. The role of M protein in the mature virion is not known.

1.6.3. E glycoprotein

It is a major virion envelope glycoprotein. E-protein acts as the target and as the modulator of host immune response. Most of the molecular markers for pathogenicity have been localized in the E gene.

1.7. The Nonstructural Proteins

NS1 contains 12 completely conserved cysteine residues. The 6 conserved disulfide bridges are likely to be critical determinants of antigenicity and function. In infected cells in vitro, NS1 is detected intracellularly, on the surface, and secreted in copious amounts into the medium.[3]

NS1 elicits humoral and cellular immune response. Passive transfer of NS1 antibodies can confer protection against DENV infection in experimental animals and NS1 has been evaluated as a candidate vaccine that can induce a protective immune response in experimental animals against homologous

challenge with DENV. NS1 plays a role in pathogenicity, due to epitopes that mimic those of endothelial cell surface molecules. Antibodies to such shared epitopes induce endothelial cell damage and elicit inflammatory cytokines that may play a role in the hemorrhage associated with dengue infection.

The non structural proteins are involved in the RNA replication and virus assembly. NS2b, NS4a and NS4b proteins are enzyme cofactors. NS3 and NS5 proteins are viral proteases.[3]

1.8. The vector

Aedes (Stegomyia) mosquito species such as. *Ae. aegypti, Ae. albopictus, Ae. polynesiensis* and some members of *Ae. scutellaris* act as vector. Among these *Aedes aegypti* is the commonest.[3] The viruses are maintained in a mosquito-human-mosquito cycle, with periodic epidemics occurring at 3 to 5 year intervals.

The larval stages are found mostly in artificial containers with water around human dwellings where they mature into adults. The people, rather than mosquitoes, transmit the virus within and between communities.[1]

The mosquitoes become infected when they imbibe blood from a viremic host. In the mosquito the virus infects epithelial cells lining the midgut wall and subsequently spreads to other organs after 8 to 12 days, transmitting the virus to other humans during subsequent feeding.

The *Ae.aegypti* female feeds on several persons to fulfill a single blood meal and transmitting the virus to multiple persons within a short period of time, making it an efficient epidemic vector.[7]

1.9. Factors influencing transmission

Uncontrolled urbanization, expanding urban population, poverty, ineffective public health infrastructure, faster modes of transportation, globalization of trade and increased international travel have all been implicated as factors leading to the spread of dengue around the world. Rapid urbanization is probably the single most important contributing factor resulting in poor sanitation and lack of potable water supply. So the residents have resorted to using containers to store water which often ends up as breeding sites for the *Ae. aegypti* vector.[7]

1.10. Host immune response

After an incubation period of 4 -10 days, infection in humans produce a wide spectrum of illness, most infections being asymptomatic.

During the acute phase of illness viremia lasts for 5 days and immune response clears the virus via neutralizing antibodies or by activation of T lymphocytes.

Infection with any one serotype provides lifelong immunity against homologous reinfection. The subsequent infection by different serotype increases the risk of development of severe manifestations.[1]

Severe dengue can also occur in infants born to Dengue-immune mothers.[8]

1.11. Pathogenesis and pathophysiology

The immune system plays a role in the pathogenesis of DHF/DSS and secondary dengue infections. Basically, at least two theories have been presented to explain the severity of DHF. According to the first theory of Antibody dependent enhancement (ADE),(Fig -2) previous antibodies of a specific serotype of dengue virus bind to different dengue virus serotype producing non neutralized antibody–virus complexes. These complexes bind to macrophages which lead to activation of T cells from previous presentation to Major Histocompatibility Complex molecules (MHC) and cytokines like interferon-γ, Tumor Necrosis Factor, IL1, IL6, IL8 are produced. Additionally, Vascular Endothelial Growth Factor (VEGF) is produced by monocytes and endothelial cells produces changes in vascular permeability due to functional alteration in vascular integrity resulting in selective leakage of plasma in the pleural and peritoneal cavities, contracted intravascular volume and shock in severe cases.[9]

According to the second theory, specific genotypes of dengue virus which are more virulent could be the cause of more severe symptoms of dengue.

Epidemiological studies show the relationship between severity of epidemics and previous serological conditions of the population affected. Sequence of infection with DENV-1 virus followed by DENV-2 has been

demonstrated to correlate with high rates of DHF.[10]

Severity of dengue correlates with high viral load, secondary dengue infection, DENV-2 virus type, virulence of the strain and the genetic difference in the hosts.[11]

1.12. Clinical presentation

Dengue infection may be asymptomatic or may cause dengue fever (DF) or dengue haemorrhagic fever (DHF) including dengue shock syndrome (DSS).[1]

According to WHO, a clinical case of Dengue Fever is an acute febrile illness with frontal headache, retroocular pain, muscle pain, joint pain and rash; even though other signs and symptoms could also be present (such as lymphadenopathy, petechiae, nausea, hepatomegaly and different types of hemorrhagic manifestations).

A probable case of DF is defined as cases with fever and two or more of the following: headache, retro-orbital pain, myalgias, arthralgias, rash, hemorrhagic manifestation and supportive serology (HAI,IgG,IgM tests) or occurrence at the same location and time as other confirmed cases of dengue.[7]

A confirmed case of DF is defined as cases with isolation of dengue virus from serum or autopsy sample; or demonstration of fourfold change in reciprocal IgG or IgM antibody titers in paired serum samples; or demonstration of dengue virus antigen in autopsy tissue, serum or cerebro-spinal fluid samples by immunohistochemistry, immunofluorescence or ELISA; or detection of virus

genome sequence in autopsy tissue, serum or cerebro-spinal fluid samples by RT-PCR.

Dengue Haemorrhagic Fever

Dengue Hemorrhagic Fever case has to have all the four following criteria: Fever, hemorrhagic tendencies (including Tourniquet test), low platelet count ($1x10^5$ per mm^3 or less) and plasma leakage (hematocrit >20%, signs of plasma leakage: pleural effusion, ascites and hypoproteinemia).[1]

DHF is classified into four grades of illness based on severity (WHO 1997)

Grade I is mild; Fever, haemorrhagic manifestations and evidence of plasma leakage.

Grade II is more severe: Grade I plus spontaneous bleeding.

Grade III is Grade II plus signs of circulatory failure.

Grade IV is Grade III plus profound shock.

The Grade III and IV is Dengue shock syndrome.

With the recent revision of dengue classification scheme by World Health Organization, patients are classified as having either dengue or severe dengue. Patients who recover without major complications are classified as having dengue. Those who have plasma leakage resulting in shock, accumulation of serosal fluid causing respiratory distress, or both; severe bleeding; and severe organ impairment are designated as severe dengue cases.

1.13. Complications

Complications in Dengue infections occur usually due to prolonged shock leading to metabolic and electrolyte disturbance with various manifestations like febrile seizure in young children, encephalopathy, severe bleeding as a result of disseminated intra vascular coagulation and multi organ failure such as hepatic and renal dysfunction. The treatment related complications due to fluid volume overload can occur. The other complications are aseptic meningitis, encephalitis, myocarditis, haemolytic uremic syndrome, pancreatitis, transverse myelitis, macular haemorrhage and optic neuritis.[3]

1.14. Differential diagnosis

It includes influenza, measles, rubella, chikungunya, enteric fever, malaria, viral hepatitis, leptospirosis, rickettsial diseases and bacterial sepsis. The infections that may clinically mimic dengue are other viral hemorrhagic fevers, and arboviral infections that may be accompanied by rash and resemble Dengue fever include, O'nyong nyong, Sindbis, Mayaro, Ross River and West Nile viruses.[3]

1.15. Laboratory diagnosis of dengue infection

Rapid and accurate dengue diagnosis is of paramount importance for epidemiological surveillance, clinical management and vaccine trials. Epidemiological surveillance requires early determination of dengue virus infection during the outbreak for urgent public health action towards control and detection of circulating serotypes/genotypes during the inter-epidemic periods

for use in forecasting possible outbreaks. Clinical management requires early diagnosis of cases, confirmation of clinical diagnosis and for differential diagnosis from other flaviviruses /infection agents.

The following laboratory tests are available for diagnosis of dengue infections.

1. Virus isolation / serotypic/genotypic characterization
2. Viral nucleic acid detection
3. Viral antigen detection
4. Immunological response based tests- IgM and IgG antibody assays.

During the early stages of the disease (up to six days of onset of illness), virus isolation, molecular techniques or antigen detection is used, after which immunological tests are used for diagnosis.

1.15.1. Virus isolation

Viral isolation is the most sensitive way to detect dengue virus. The techniques used are:

1. Mosquitoes (pool of 15 to 20) are inoculated either with serum, or plasma, or pleural fluid or other sterile body fluid. After incubation virus infection is confirmed by immunofluorescence. This is the most sensitive isolation technique.
2. Inoculation of mammalian or insect cell cultures (usually C6/36) is other common method used for viral isolation. The presence of the viruses is

confirmed by cytopathic effect or plaque formation assay. RNA detection and immunofluorescence also can confirm the infection.

3. Intracranial inoculation of sucking mice is used to isolate dengue virus; either encephalitis signs or antigen in brain tissue are evidence of infection.

The higher limitation of the viral isolation is that it is time consuming, and also there is high cost associated with use of the cell culture method.

1.15.2. Viral nucleic acid detection

Molecular diagnosis is more sensitive and rapid than virus isolation. In recent years, a number of RT-PCR assays like RT-PCR, Nested PCR, one step multiplex PCR, Real time RT-PCR and Isothermal amplification method have been reported. Recently, Loop Mediated Amplification (LAMP) PCR method has been developed, which promises an easy-to-do and less expensive instrumentation alternative for RT-PCR and real-time PCR assays. However, molecular diagnoses are costly, require specialized laboratory equipments and experienced technicians.

1.15.3. Viral Antigen detection

The NS1 gene product, needed for replication and viability is formed by all flaviviruses. The protein is produced by infected mammalian cells and the antigen appears as early as the 1st day of fever declining to untraceable levels by 5–6 days.

NS1 antigen is present in high concentrations for up to six days after the onset of the illness in both primary and secondary dengue infections. However,

the kits do not differentiate between the serotypes. Besides providing an early diagnostic marker for clinical management, it may also facilitate the improvement of epidemiological surveys of dengue infection.

The newer diagnostic assays for E, NS3 and NS5 antigen detection allows the diagnosis of dengue with higher sensitivity during acute phase of infection.[11]

The Immunoperoxidase and avidin-biotin enzyme assays allow detection of dengue virus antigen in acetone-fixed leucocytes and in snap-frozen or formalin-fixed tissues collected at autopsy.

1.15.4. Viral Antibody detection

The use of immunological test is based in the immune response after being exposed to the Dengue virus. Antibody response to infection leads to the appearance of different types of immunoglobulins. Detection of immunoglobulin M (IgM) and immunoglobulin G (IgG), each one or in combination, are used in the diagnosis of dengue infection. A definitive diagnosis requires a pair of samples, which need to be collected during the acute and convalescence phase.

During primary infection, the IgM antibodies are detectable by days 3 to 5 after the onset of illness, rise quickly by about two weeks and decline to undetectable levels after 2–3 months. IgG antibodies are found at low level by the end of the 1st week, increase subsequently and remain for many years.[7]

During secondary dengue infection, antibody titres rise rapidly. IgG antibodies are demonstrable at high levels, even in the initial stage, and persist

lifelong. IgM antibody levels are significantly lower in secondary infection cases. Hence, a ratio of IgM/IgG is commonly used to differentiate between primary and secondary dengue infections.

Several methods like Enzyme linked immunosorbent assay (ELISA), Haemagglutination inhibition test (HAI), complement fixation test and neutralization test are used for the serological diagnosis of dengue.

Among those, IgM/IgG capture ELISA is the most commonly used serological technique for routine diagnosis due to its high sensitivity, specificity, simplicity, and feasibility for automation.HAI test has recently become less popular due to the inherent disadvantages of the test.

A number of commercial rapid format serological tests have become available in the past few years, some of these producing results within 15 minutes. But the accuracy of most of these tests is uncertain as they have not been validated yet. Rapid tests can yield false positive results due to cross-reaction with other flaviviruses, malaria parasite, leptospires and immune disorders such as rheumatoid and lupus. But in an outbreak situation, if more than 50% of specimens test positive when rapid tests are used, dengue virus is then highly suggestive of being the cause of febrile outbreak.[1]

1.16. Interpretation of dengue diagnostic test[1]

Highly suggestive of dengue infection

One of the following:

1) IgM positive in a single serum sample.

2) IgG positive in a single serum sample with a HAI titre of 1280 or greater.

Confirmed dengue infection

One of the following:

1) RT-PCR positive.
2) Virus culture positive.
3) IgM seroconversion in paired sera.
4) IgG seroconversion in paired sera or fourfold IgG titre increase in paired sera.

1.17. Treatment and prevention

Proper and early treatment can relieve the symptoms and prevent complications and death. In the early febrile phase, since it is not possible to distinguish Dengue fever from Dengue Haemorrhagic Fever clinically, the treatment is symptomatic and supportive care only. There is no role for antibiotics and steroids. Timely intravenous fluid therapy can prevent shock and/ or lessen its severity. In case of severe bleeding or reduction in haematocrit fresh blood transfusion must be required.[1]

There are three approaches to prevent dengue disease,

1. To detect the cases and the virus in early periods of viral activity by epidemiological surveillance either to reduce or prevent the impact of the epidemic.
2. To halt the infectious chain reducing or eliminating the vector mosquito before transmission, during and after rainy season.

3. To block the virus action inside the human hosts with use of vaccine to induce specific immunization. With a mixture of environmental management, health education and community participation the disease can be eradicated.[1]

Multicentric phase 2, 3 trials are in progress to determine the efficacy of ChimeriVax (Sanofi Pasteur), a tetravalent formulation of attenuated yellow fever 17D vaccine strains expressing the dengue virus prM and E proteins. Long-term follow-up will be essential to assess whether waning vaccine-elicited immunity leads to severe outcomes on subsequent natural infection. Live attenuated dengue viruse vaccines and recombinant subunit vaccines are in the row.[12]

Since differentiation of dengue from other febrile illnesses in acute phase is clinically difficult, definitive and early diagnosis relies on laboratory tests which is important in the therapeutic intervention to decrease morbidity and mortality.

Due to the rising importance of early and prompt dengue infection detection, this study was aimed to analyze the importance of NS1 antigen detection in early diagnosis of dengue and its correlation in primary and secondary infection.

2. AIM AND OBJECTIVES

1. To study the prevalence of dengue in paediatric age group.
2. To study the importance of NS1 antigen in the diagnosis of dengue infection.
3. To demonstrate the importance of paired serum samples in the interpretation of dengue infections.

3. REVIEW OF LITERATURE

Dengue, a serious mosquito borne infection in humans occurs during rainy season and affects over hundred million people every year with high death rate in children. A primary dengue infection provides lifelong immunity against the infecting serotype and a brief protection against infection by other DENV serotypes.[2] But this increases the probability of an individual developing DHF when infected by a second heterologus DENV serotype due to antibody dependant enhancement. Till date there is no effective antiviral drug or vaccine for this disease. Prevention, early diagnosis and supportive care are the mainstay of treatment in dengue infection.

Dengue virus was first isolated by Ren Kimura and Susumu Hotta in Japan in 1943. A few months later Albert Bruce Sabin and Walter Schlesinger isolated dengue virus from Hawaii.[13] Of the four serotypes DENV 2 and DENV 3 are associated with severe disease.

3.1. Global prevalence of dengue

Historically, there are at least three different sources providing report about the first dengue case; chronologically, a report from China provided the first description of a disease compatible with dengue in 610 A.C. The second report that probably registered the source of dengue outbreak was in West French Indies in 1635. Finally, and usually the most cited dengue report was from Philadelphia in the summer of 1779.[7]

The first epidemic of DHF occurred in Manila, Philippines, in 1953 to 1954 and within 20 years, the disease in epidemic form had spread throughout Southeast Asia. By the mid-1970s, DHF had become a leading cause of morbidity, since then the disease has increased more than four fold.

During the 1980s and 1990s epidemic Dengue transmission intensified, and there was a global resurgence of Dengue fever, with expanding geographic distribution of both the vectors and the viruses and the emergence of DHF in many countries. In 1998 alone, more than 1.2 million cases were reported to the World Health Organization, with south-east Asia, the western Pacific and more recently the America being the most affected regions.

In the South East Region of Asia from 2000 to 2005, the highest cases of dengue were in Maldives, Thailand, Sri Lanka and Indonesia.[1]

3.2. Dengue seroprevalence

In Indonesia dengue serotype antibody prevalence was estimated twice during a one year interval. In the beginning a study conducted among 1,837 children aged 4 to 9 years in 1995 showed 56.1 % of the children were positive to dengue antibodies.[14]

In Vietnam, Thai et al[15] (2005) reported sero-prevalence of 65.7 % in schoolchildren aged 7 to 14 years.

A prospective study made by Balsameda et al[16] (2006) in Nicaragua among children 4 to 16 year old and reported that the overall prevalence was 91%, increasing from 75% at age 4 to 100% at age sixteen.

In Maracay of Venezuela, a prospective study performed on schoolchildren aged 5 to 13 years showed the prevalence of anti dengue antibody to be 51 % ; amongst which 30.1 % tested with immune response to one serotype, and 20.9% to two or more serotypes. The highest antibody prevalence was for DENV-2 (14.2%) followed by DENV-1 (13.4%). Among the previously seronegative children, 25.6% seroconverted and 26% of the children seroconverted with secondary infection.[17]

3.3. The Indian Scenario

Dengue haemorrhagic fever was first reported in 1963 from Calcutta. The next major epidemic of DHF occurred in New Delhi in 1996 when 10,252 cases and 423 deaths were reported with the proportion of DHF to DF being very high.

Out of 35 States / Union-territories in the country 29 have reported dengue cases mainly due to DENV 2 and deaths due to DENV 3.

During the post epidemic period of 1997, DENV-1 activity was seen in New Delhi. The cases and deaths were low till 2002 but again it raised in 2003, when another outbreak occurred where all four serotypes were co-circulating with a predominance of DENV 3. [5]

In 2004, DENV 1 was found to be circulating and in 2005 DENV 3 emerged as the predominant serotype.

In 2005, the cases and deaths showed threefold increase compared to 2004.

PM Ukey et al (2010)[18] in his study observed that the sero prevalence in Central India was 31.3% from the period of 2005 to 2006. In Andhra Pradesh in South India, Neeraja et al[19] tested 260 dengue suspected cases during 2004 outbreak and reported sero positivity in (81%) patients.

3.4. Dengue in Tamil Nadu

The first evidence of Dengue fever in the state was documented from Vellore District, Tamil Nadu in 1956.[20] In South India particularly in Tamilnadu DF and DHF have been well documented.[21]

Of the 30 districts in Tamilnadu, dengue cases have been reported from 29 districts between 1998 and 2005 which include DF / DHF outbreaks in Chennai 2001, Nagerkoil 2001 and Thiruchirapalli 2003 and Dengue fever outbreaks in Krishnagari and Dharmapuri Districts in 2001. Total of 128 cases and 5 deaths were reported in 1998 which increased to 1600 cases and 12 deaths in 2003 and 1150 cases and 8 deaths in 2005.Dengue cases have not been reported from Nilgris District probably due to high altitude.[22]

In Chennai, uncomplicated Dengue fever (DF) was prevalent among children and adults and DHF has been largely restricted to infants and children. Since 2005 Chennai is witnessing DHF among young as well as elderly adults.[23]

During July 2007, Dengue fever suspected cases were reported from two rural areas namely O.Alankulam and P.Alankulam near Madurai, TamilNadu.[24]

3.5. Dengue season

Due to the rise of the larval population in the rainy season epidemics of dengue tend to occur during that time. The rainy season provides the required temperature and humidity for the propagation of virus in mosquitoes. The temperature of the environment also influences the time taken for the virus to multiply in mosquito, especially rainy season being favourable for the virus to cause early acute viraemia. Gupta E et al[25] observed that the seasonal trend of dengue virus infection is reflected by the peak of positive cases observed during post monsoon season.

Barbazan et al[26] (2002) in Thailand observed in their study that dengue was more prevalent during and after rainy season, when vector breeding is maximal.

R.K.Ratho et al[27] (2006) noted that, between 1996 to 1999 large cases of dengue occurred from September to November.

3.6. Dengue in rural area

In the 1960s and 1970s dengue was mainly an urban disease but with the usage of piped water supply by the rural communities, it made it entry into rural areas of South India.[28]

S.C.Tewari et al[6] (2004) concluded from their entomological study that villages in South India are known for the hyper-endemicity of dengue where all the four serotypes are circulating and prone for outbreak.

Arunachalam et al[29] (2004) from their study reported dengue cases for the first time in a rural area of Kurnool District, Andhra Pradesh.

3.7. Dengue in children

WHO (1997) estimation of dengue data suggest that annually hundred million cases of dengue fever and fifty thousand cases of dengue haemorrhagic fever (DHF) are occurring worldwide with a fatality rate of 0.5%–3.5% in Asian countries. Of those with DHF, ninety percentages are children less than 15 years of age.

Maria Guzman et al[2] in 2002 analysed the sero epidemiological data from 1981 epidemic of Cuba and reported that children, aged 3 and 4 years, with secondary DEN-2 infections were found to have a high death rate. The death rate for children aged 3-14 years was 14.5-fold higher than in young adults aged 15-39 years.

In Thailand, data from 1974 to 1993[30] showed that dengue was common in children aged less than 15 years of age and the incidence rates among children hospitalized with dengue have been consistently highest in the 5–9 year age group.

Rosario et al[8] in his study observed that the infants in endemic area are more prone to develop dengue. In 2007, the incidence of asymptomatic dengue infections during infancy was 103 per 1,000 person-years which were six times higher than symptomatic cases. DHF was known to occur in 0.5 per 1,000 persons who were in the age of 3–8 months, and it was not present by age 9

months because younger infants are more likely to have protective levels of maternally derived anti-DENV antibodies.

3.8. Risk factors

Risk factors include the infecting type of the virus, virulence of the virus, gender, immunological status and hereditary background of the host.[31]

Death rate and hospitalization rates because of DHF/DSS are maximum following a secondary DENV 2 infection.

Neeraja et al[19] in 2012 showed the male female ratio to be 2:1.Ole Wichman et al[32](0.96:1) and Kabra et al[33] reported that DHF to be more severe among females.

A study among 165 patients by Narayanan et al[34] showed that the occurrence of male, female dengue cases were 52.4%, 47.6% respectively. Gomber et al[35] found it to be 56% and 44%, and Aggarwal et al[36] as 60% & 40% males and females respectively.

Malnutrition appears to be an uncommon risk factor for DHF. It is common among patients suffering from diabetes mellitus and bronchial asthma.[37]

3.9. Clinical profile

Ratgeri et al[38] (2005) observed clinical features and lab findings such as fever (100%), vomiting (82%), pain abdomen (61%), headache (22%), Gastro intestinal bleeding (22%), petechiae (18%), WBC count less than 5000/cumm

(26%), platelet count below 1 lakh/µl (82%), pleural effusion (70%) and ascites as USG finding (54%) of cases in his study.

Aggarwal et al[36] (1997) observed the following findings. Among 134 cases, fever (93%), vomiting (68%), abdominal pain (49%), haematemesis (39%), epistaxis (36%) and skin bleeds in 33% of cases. WBC counts below 4000/cumm were observed in 15% of cases, platelet below 50,000/µl in 69% and haematocrit more than 40% in 18% of cases.

Narayanan et al[34] (2002) among 59 patients observed, fever 98.3%,vomiting 83%,headache 28.8%, retro orbital pain in 11.9%, abdominal pain 23%,bleeding 66.1% ,rash 8.5%, fall in haematocrit in 24.1% and platelet count below 1 lakh in 39% of cases.

3.10. Mortality

DENV infections cause illness in tens of millions each year throughout the tropics and subtropics, severe morbidity in approximately 2 million persons/year, and approximately 20,000 deaths/year. The mortality of dengue from various studies reported were 12-13% (Kabra et al,[33] 6% (Aggarwal et al),[36] 4.8% (Gomber et al),[35] 3.3% (Narayanan et al),[34] 5.2 % (Neeraja et al)[19] and nil mortality (Kabilan et al 2001).[39]

3.11. Detection methods

NS1 antigen

Dengue NS1 protein is found to be circulating in early period of illness and does not have cross reaction with Japanese encephalitis or yellow fever.

NS1 antigen detection is an effective test for the diagnosis of dengue during the early stage. NS1 antigen is noticeable in blood from the first day after the onset of fever up to Day 9; once the clinical phase of the disease is over it is still detectable even when viral RNA is negative by RT-PCR and in the presence of IgM antibodies.

Alcon et al (2002)[40] analysed 127 panel of sera from 61 patients and determined the NS1 antigen level each day. They observed that it was 80% positive on day one,60% positive on day two,100% positive on day four and five, 80% on day six and it was hardly detected between days seven & nine and never detected after day ten.

Xu et al[41] (2006) analysed specimens from clinically suspected dengue patients and observed that NS1 antigen could be detected till the 18th day after the start of illness with increased levels on days six to ten. Both NS1 and IgM were present in the acute phase, but during first three days, NS1 antigen detection was more sensitive.

Dussart et al (2006)[42] analysed 239 acute serum samples for NS1antigen and compared with RT-PCR, which showed low sensitivity for NS1 from day 5 and suggested the possibility of immune complexes production from day 5. They concluded that NS1 antigen detection could be used for first line testing from day 0 – 4 of onset of fever for primary dengue fever and suggested the study of paired sera for analyzing the effectiveness of NS1 antigen in severe dengue disease.

Mini P.singh et al(2010)[43] has evaluated and compared NS1 with IgM for diagnosis of acute dengue virus infection in 87 patients and said that NS1 antigen had a sensitivity of 71% to 100% in patients who had fever for 3 days. They suggested that NS1 antigen should be considered as the test of choice for patients presenting with fever upto 3 days and it was not useful beyond day 4 of fever.

Kovi Bessof et al (2008)[44] conducted a study among 208 acute serum samples and concluded that NS1 detection can improve dengue virus diagnosis.

A.Shrivastava et al (2011)[45] in their study evaluated NS1 antigen detection among 91 acute serum samples and compared it with RT-PCR. Their results showed that 26% were positive for NS1 antigen and only 12% were positive by RT-PCR. They concluded that plasma viremia levels had correlation with the detection of NS1 and reported that patients who tested positive for NS1 antigen had high viremia levels and vice versa. So acute dengue cases with low viremia can be NS1 negative and the sensitivity of NS1 decreases with increasing time as the viral load comes down in these patients. NS1 has a high Positive predictive value in the diagnosis of dengue, but negative NS1 does not rule out dengue fever.

Kwoon-Yong Pok (2010)[46] in his study, compared the detection of NS1 antigen with RT-PCR in dengue diagnosis and stated that it is an effective alternative choice to RT-PCR.

Duong et al (2011)[47] conducted a study among 260 confirmed dengue patients and reported that the sensitivity of NS1 test increased when combined with MAC-ELISA.

Chua et al (2011)[48] conducted a prospective study to evaluate the sensitivity of Dengue NS1 antigen detection in 558 acute phase serum samples and compared with virus isolation, RT-PCR and IgM detection. NS1 positivity was 91.6% and the authors concluded that it was more sensitive than virus isolation.

Laurent Thomas et al (2010)[49]conducted study among 70 acute phase serum samples and reported NS1 positivity of 67.1%.They concluded that the presence of NS1 antigen correlated with plasma viral load and was not dependent on the immune status and sampling time. Increased viremia, secondary infection and severe disease are associated with NS1 positivity.

Ivani Bisordi et al(2011)[50] conducted a study among 519 serum samples and found that NS1 antigen positivity was high (99.3%) suggesting that NS1 antigen detection is highly suitable tool for early diagnosis and for monitoring the introduction and spread of dengue serotypes.

WHO stated that MAC-ELISA is the test of choice for rapid diagnosis in a single sample, collected after the fifth day of disease. The result was not considered as lab confirmation of acute dengue without a convalescent serum sample and could be interpreted as recent dengue infection.

According to Pan American Health Organization (1994) guidelines,[51] by day five of illness 80% of cases have detectable IgM antibodies and by day 6-10, 93-99% of cases have detectable IgM that may persist for over 90 days.

Chew Then Sang et al[52] in 1998 tested 148 patients acute phase serum samples and showed that 130 patients were positive for IgM with sensitivity of 88% and specificity of 91%.

The ability to detect true positives by IgM ELISA varies from 83.9% to 98.4% with a 100% chance of detecting true negatives. This range is important in secondary dengue patients where IgM antibody titers are only minimal.

Innis et al (1997)[53] observed from his study that the viremia and fever ended, as IgM started to appear. In many of the cases it appeared by the 3rd day and disappeared after one to two months. IgM positivity was 78% in acute sera and 97% in paired sera. In secondary dengue infections the increase in IgM response was slow, mild and lasted only for a short time.

Prince et al (2011)[54] analysed paired serum samples of 145 patients collected less than 30 days apart and demonstrated the seroconversion which was used to confirm and classify the infection into 83 primary and 63 secondary infections. If both IgM, IgG were positive in acute sample, it indicated that the seroconversion had already occurred.

Dong Mi Hu et al (2011)[55] study shows that anti dengue IgM antibody positivity was 42.9% on the third day and it rapidly increased to 100% by day eight of illness. The sensitivity of NS1 detection during the first seven days

ranged from 81.8% to 91.1%.A combination would be suitable in developing countries for enhanced diagnosis where dengue is endemic.

Kassim et al[56] tested 208 serum samples and the NS1 positivity was 38.5%, IgM detection rate was 36.1%, combination of antigen and antibody was 62% and concluded that this combined testing could increase the efficiency of dengue diagnosis.

Stuart D. Blackshell et al[57], suggested that combining the NS1 antigen and IgM antibody detection gave high sensitivity and specificity in the diagnosis of acute dengue infection for patients presenting at different times after fever onset.

Gowri shanker et al[58] reported in a study done during an outbreak of dengue fever that IgM antibody was positive in all the serum samples that were positive for NS1 antigen which were collected within first 5 days of infection. The sensitivity of NS1 with IgM detection was higher when compared with RT-PCR and could be used for early diagnosis.

Sathish et al[59] in his study concluded that IgM antibodies appear as early as three days of dengue viral fever and can persist up to 30–60 days. IgM antibodies are useful in providing a provisional diagnosis from a single serum sample.

In a study in Puerto Rico, most patients (80%) by day 5 of illness had detectable IgM. Almost all patients (93%) developed detectable IgM 6 to 10

days after the onset of fever, and 99% of patients tested positive between day 10 and 20. (Kuno et al 1991).[60]

Schilling et al[61] in 2004 studied the sensitivity of IgM detection in paired serum samples and correlated with virus isolation. His results showed that the samples of patients with primary dengue infection taken during days 1-3 of the disease had no IgM antibodies. During days 4-7 and after day 7, IgM antibody was detected in 55% and 94%, respectively. In patients with secondary dengue infections, less positive IgM was found in samples taken during days 4-7 (47%) & after day 7 (78%).

Anita Chakravarthi (2011)[62] conducted a study in acute phase serum samples and observed that the IgM alone were detected in 34% of the samples and NS1 antigen alone was detected in 39.7% of the samples, while 26% were positive for both NS1 antigen and IgM antibody. NS1 antigen was detectable in patient sera from Day one onwards and dengue IgM antibody was detected from Day three onward and it gradually increased in positivity toward the end of the acute illness. From this they concluded that only NS1 antigen can be used during the first two days of fever, IgM antibody after the third day of fever and no significant difference between both tests from day three to seven.

IgG antibody is usually detected from day nine of illness in primary infection. If it is seen before that it indicates secondary infection with dengue. WHO 2009 dengue guidelines says, IgG is used for the detection of recent or past dengue infections for duration of 10 months after infection but they can be

present for life time. A fourfold or greater rise in IgG antibodies in acute and convalescent sera is the criteria for recent infection. In areas where dengue is endemic, it is difficult among infants to exclude the presence of passively transferred maternal IgG antibodies or IgG antibodies acquired due to past exposure. The problem can be eliminated by confirming the absence of dengue virus IgG antibodies in the maternal serum.

Vaughn et al (1999)[63] in his study reported that the IgG capture ELISA showed good correlation with HAI assay which is the gold standard to differentiate primary and secondary dengue infections. They suggested that IgG could be used to discriminate 100% of primary and 96% of secondary dengue infections.

Miagostovich etal[64] studied IgG ELISA for dengue and concluded that IgG when analyzed by days after onset of symptoms could be reliably associated with primary or secondary infections. It can be used to characterize the immune response after flavivirus infections.

Inoues et al (2010)[65] stated that IgG detection is simple, rapid and sensitive test that can be used in the diagnosis of primary and secondary infections.

Vazquez S et al (2007)[66] has shown in his study that ELISA inhibition method is used to look for the presence of IgG in serum sample, blood plasma and blood samples absorbed onto filter paper permitting the classification into primary or secondary dengue infection.

3.12. Significance of testing paired serum

Hunsperger et al (2009)[67] concluded that although demonstration of IgM seroconversion in the convalescent sample is taken as a confirmation, presence of IgM in a single specimen collected from a patient with clinical findings consistent with dengue, gives a presumptive diagnosis.

Andrew Falconer et al (2006)[68] conducted a study among 100 paired serum samples from suspected dengue cases and reported that 37 cases were current flavivirus infections if they showed greater than 4-fold increases in DENV-specific IgM or IgG between their acute and convalescent phase serum samples.

3.13. Primary and Secondary infections.

Primary infection was defined as an IgM positive/IgG negative in the first specimen and an IgM negative/IgG negative in the first specimen becomes IgM-positive/IgG-positive in the second specimen. Secondary infection was defined as an IgM-negative/IgG-positive first specimen and an IgM-positive/IgG-positive second specimen.

Primary dengue infections usually manifests as a simple fever indistinguishable from other viral infections. They are less likely to develop severe disease and shock syndrome whereas secondary infections tend to be associated with higher morbidity and mortality especially in children. Reliable tests for dengue differentiation are essential for patient management and also to predict the disease progression and assess mortality especially in severe cases.

3.14. Correlation of NS1 in primary and secondary infections

Duong et al[47] noted high NS1 Antigen positivity rate in dengue fever than DHF/DSS, in primary than secondary infection.

Kumarasamy et al[69] reported that the NS1 detection sensitivity rate was higher in primary dengue (97.3%) than in secondary dengue (70%).

S D Sekaran et al (2009)[70] in their study among 93 acute serum samples found that NS1 detection rate was higher in the acute primary dengue (100%) than in the acute secondary dengue (53.5%) serum samples indicating that the Dengue antigen assay is a more sensitive assay in the primary acute phase when IgM is not detectable.

Kwoon-Yong Pok et al[46] observed decreased sensitivity of NS1 antigen testing in secondary dengue infections. Though it was an effective assay, this was a disadvantage of NS1 antigen testing.

Young et al (2000)[71] observed that high levels of NS1 were found in acute DENV 2 secondary infections and not in convalescent sera. In contrast, NS1 was not found in both acute and convalescent sera of primary infections. The presence of high levels of NS1 contributes significantly to the formation of the circulating immune complexes that play a major role in the pathogenesis.

A study conducted by Koraka et al[72] showed that in endemic areas NS1 antigen in serum samples was low due to the presence of NS1 antigen- anti NS1 antibody complexes. These complexes were detected much more frequently in patients with secondary dengue infections where DHF/DSS is more frequent.

To overcome the problem of low NS1 positivity in acute secondary infection, acid dissociation of the complexes was suggested, which increased the sensitivity of NS1 detection by 25%. Lapphara et al[73] observed an increase of sensitivity in NS1 detection from 63.2% to 72% with immune complex dissociation by acid treatment.

The haemorrhagic symptoms are more severe in secondary dengue infections. The test to discriminate the primary and secondary infection, was HAI which was suggested by WHO. But it was very difficult to perform. A.Chakravarti et al[62.] reported that the ELISA itself is an absolute alternative for the HAI the gold standard suggested by WHO to discriminate between the primary and secondary dengue infection.

4. MATERIALS AND METHODS

4.1. Study place

This study was conducted at Tirunelveli Medical College Hospital, Tirunelveli, among the dengue suspected paediatric cases admitted in the hospital.

4.2. Study period

The study period was from August 2011 to January 2012.

4.3. Ethical consideration

Written consent to participate in the study was obtained from the children's parents or guardians after explaining the full study. This study was reviewed and approved by Institutional Ethical Committee, Tirunelveli Medical College, Tirunelveli before the commencement of the study.

4.4. Study population

A total of 110 blood samples were collected from sixty five children, 45 had paired sample.

4.4.1. Inclusion criteria

1. Paediatric patients (0-12years)
2. Children admitted with acute febrile illness with two or more of the following features of WHO criteria- headache, myalgia, retro orbital pain, rash, haemorrhagic manifestations and thrombocytopenia.

4.4.2. Exclusion criteria

1. Febrile cases without the above features
2. Haemorrhagic conditions without symptoms of Dengue fever.
3. Patients with clinical evidence of respiratory tract infection, urinary tract infections, acute diarrheal disease, injury, sepsis, or other apparent causes of fever were excluded.

4.5. Sample collection

Clinical data was collected from all children admitted with fever in the paediatric ward. Blood sample was taken from children who had fever with one or more of the following features like thrombocytopenia, haemorrhagic manifestations like epistaxis, bleeding gums, haematemesis, malena, petichial haemorrhages, features of leaky capillaries like pleural effusion, ascitis seen on X'ray or ultrasound and haematuria on urinalysis.

Blood samples were collected from the children with above mentioned clinical picture of dengue infection after taking written consent from their parents. Three to 4 ml of whole blood was drawn by the intravenous route with aseptic precautions & transferred to a sterile leak proof glass container. The blood samples were transported to the lab immediately with proper labeling (name of the patient, identification number and date of collection)

Patients were advised to come to the paediatric outpatient clinic after 2 weeks of discharge for review and the second sample was collected.(convalescent sample)

4.6. Sample processing and storage

In the lab, the samples were kept at room temperature (20-25^{o}C) for at least 30 minutes. Then serum was separated by centrifuging at 2000 rpm for 3 minutes and stored at -20^{o}C until testing, in a sterile vial. Icteric or lipemic sera exhibiting haemolysis were rejected. Proper biomedical waste management was followed throughout the study.

For all the 65 samples collected in the acute phase, NS1 antigen and IgM ELISA was performed.

For the 45 samples collected in the convalescent phase IgM ELISA was performed. For the same 45 samples IgG was tested in the acute sample (collected earlier) and convalescent sample.

4.7.ELISA kits

Dengue Early ELISA for NS1 Antigen detection, IgM Captue ELISA, IgG Capture ELISA were purchased from Panbio pty Ltd, Brisbane,Australia. The kits were stored at 2-8^{0} C.

4.8.Equipments

Deep Freezer (-20^{O}c), Incubator, ELISA washer and reader (Bio Rad, France), Accurate micropipettors with disposable pipette tips (5-1000µl capacity)

4.9. Methods

All the samples were tested for NS1 antigen, IgM and IgG in the Microbiology laboratory, Tirunelveli Medical College Hospital, Tirunelveli.

4.9.1. Dengue Early ELISA for NS1 Detection

4.9.1a. Principle

NS1 antigen, when present in the serum, binds to anti-NS1 antibodies coated on the surface of the micro wells. Horsh Radish Peroxidase conjugated Anti NS1 Monoclonal antibody hydrolyses the Tetra Methyl Benzidine substrate and colour development indicates of the presence of dengue NS1 antigen in the samples.

4.9.1b.Serum predilution

All the reagents provided in the kit and the samples were brought to room temperature.

The controls, positive and negative, Cut-off calibrators and the samples were diluted in test tubes. To 75 mic.lit of serum, 75 mic.lit of sample diluent was added and mixed. The final dilution was 1:2.

4.9.1c.ELISA procedure

1. 100mic.lit of diluted samples and controls (one positive control, one negative control, 3 cut off calibrators) were added to their respective microwells.
2. The microtitre plate was covered and incubated for 1hour at 37 deg. Centigrade.
3. After washing 6 times by diluted wash buffer, 100µl of HRP conjugated Anti NS1 Mab is added.
4. The plate was covered and incubated at 37 ^{o} C. for 1 hour.

5. The assay plate was washed 6 times. After that 100µl TMB per well was added and incubated for 10min at room temperature.
6. 100mic.lit stop solution was added to stop the reaction and the absorbance was read at 450nm and 600-650nm.

The average value of the three cutoff calibrators were calculated and multiplied by 0.66 to get the cutoff value.

The absorbance OD of the sample was divided by the value got in the previous step to get the index value.

Panbio units were calculated by multiplying the index value by 10.

4.9.1d.Interpretation of results

Index value	Units(Panbio)	Results
Less than 0.9	Less than 9	Negative
0.9 to1.1	9 to11	Equivocal
More than 1.1	More than 11	Positive

4.9.1e.Test validity

Calibration factor: 0.66

Negative absorbance: <0.300

Cut-off value: $\geq$ 1.5 x Negative absorbance

Positive control/cutoff Ratio: 2.0-9.0

4.10. Dengue IgM Capture ELISA – MAC ELISA

4.10.1a.Principle

IgM antibodies, if present, combine with anti IgM antibodies coated on the surface of the micro wells. HRP conjugated monoclonal antibody-concentrated pool of dengue 1-4 antigen complex hydrolyses the TMB substrate and colour development indicates of the presence of IgM antibodies in the test sample.

4.10.1b.Serum predilution

All the reagents provided in the kit and samples were brought to room temperature.

The positive control, negative control, cutoff calibrators and the samples were diluted (1:100) in test tubes. To 10 mic.lit of serum, 1ml sample diluents was added and mixed well.

4.10.1c.ELISA procedure

1. Ten microlitre of antigen was added to 2.5ml of antigen diluents and mixed.
2. The diluted antigen was mixed with an equal volume of MAb Tracer (Horse Radish Peroxidase conjugated Monoclonal Antibody Tracer) in a separate test tube and left at room temperature.
3. 100 mic.lit samples and controls (one positive control, one negative control, 3 cut-off calibrators) were added to the assay plate (anti human

IgM coated micro wells). The plate was covered and incubated for 1 hr at 37 deg. Centigrade.

4. The assay plate was washed 6 times. 100mic.lit of antigen – Mab solution was transferred per well to the assay plate.
5. The plate was covered and incubated for 1hr at 37 deg C.
6. The assay plate was washed 6 times. After that 100mic.lit TMB per well was added and incubated at room temperature for 10 minutes.
7. The reaction was stopped with 100 mic.lit stop solution and the absorbance was read at 450nm and 600-650nm.

The average value of the three cutoff calibrators were calculated and multiplied by 1.14 to get the cutoff value.

The absorbance OD of the sample was divided by the value got in the previous step to get the index value.

Panbio units were calculated by multiplying the index value by 10.

4.10.1d.Interpretation of results

Index value	Units (panbio)	Results
Less than 0.9	Less than 9	Negative
0.9 to1.1	9 to11	Equivocal
More than 1.1	More than11	Positive

4.10.1e.Test validity

Calibration factor: 1.14

Negative absorbance :< 0.400

Cut-off value :≥ 1.5 x Negative absorbance

Positive control/cut-off Ratio: 1.1-7.0

4.11. Dengue IgG capture ELISA

4.11.1a.Principle

IgG antibodies, if present; combine with anti IgG antibodies coated on the surface of the micro wells. HRP conjugated monoclonal antibody- concentrated pool of dengue 1-4 antigen complex hydrolyses the TMB substrate and colour development indicates of the presence of IgG antibodies in the test sample.

Serum antibodies of the IgG class, when present, combine with anti-human IgG antibodies coated on the surface of the polystyrene micro wells. A concentrated pool of dengue 1-4 antigens is diluted with diluents. HRP-conjugated monoclonal antibody is added to the diluted antigen allowing the formation of antigen MAb complexes. After washing, complexed antigen MAb is added to the assay plate. These Antigen-MAb complexes then bind to the serum dengue specific IgG antibodies. After incubation the micro wells are washed and a colourless TMB chromogen is added. The substrate is hydrolyzed by HRP if present, and the chromogen turns blue. After stopping the reaction with acid, the TMB turns yellow. Colour development is indicative of the presence of anti-dengue IgG antibodies in the test samples.

4.11.1b.Serum predilution

The positive control, Negative control, cutoff calibrators and the patients samples were diluted (1:100) in test tubes. To 10µl of serum 1000 µl sample diluents was added and mixed well.

4.11.1c.ELISA procedure

1. Antigen dilution 1:250. For this 10 mic.lit of antigen was diluted with 2.5ml of antigen diluent. This was sufficient for 5 strips. (0.5ml of diluted antigen required per strip)
2. The diluted antigen was mixed with an equal amount of MAb Tracer in a separate test tube and left at room temperature for 1hr.
3. 100µl of diluted samples and controls were added to their respective wells of the assay plate. The plate was covered and incubated for 1hr at a temperature 37 deg C.
4. The assay plate was washed 6 times with diluted wash buffer and 100 mic.lit of antigen-MAb solution, prepared in step- 2 was transferred to each well of the assay plate.
5. The plate was covered and incubated for 1hr at 37 deg C.
6. The assay plate was washed 6 times. 100 mic.lit TMB per well was added and incubated for 10 min at room temperature.
7. The reaction was stopped with 100mic.l stop solution and the absorbance of each well was read at 450nm with reference filter of 600-650nm.

The average value of the three cutoff calibrators were calculated and multiplied by 1.00 to get the cutoff value.

The absorbance OD of the sample was divided by the value got in the previous step to get the index value.

Panbio units were calculated by multiplying the index value by 10.

4.11.1d.Interpretation of results

Index value	**Panbio Units**	**Results**
Less than1.8	Less than18	Negative
1.8 to 2.2	18 to 22	Equivocal
More than 2.2	More than 22	Positive

4.11.1e.Test validity

Calibration factor: 1.00

Negative absorbance :< 0.300

Cut - off value: $\geq$ 1.5 x Negative absorbance

Positive control/cut- off Ratio: 1.1-4.0

Statistical analysis

Results were analyzed for the parameters like mean, median and percentages. Parametric tests like ‘Z’,‘t’ and non – parametric test like ‘χ2’ test, logistic regression test were used analyze the differences wherever applicable. Statistical analysis was done using SPSSversion16.software.

5. RESULTS

5.1 The Study Group

A total of 65 children admitted in the paediatric ward of Tirunelveli medical college hospital with a clinical suspicion of dengue infection were included in the study. The period of study was from August 2011 to January 2012. The study was completed before the recent outbreak of dengue at Tirunelveli district.

5.2 Age and gender of the study group

Table –1 Age and gender wise distribution of cases under study

S.No	Age (years)	Male	Female	Total
1	**0-4**	10(67%)	5(33%)	15(23%)
2	**5 – 8**	17(61%)	11(39%)	28(43%)
3	**9 – 12**	14(64%)	8(36%)	22(34%)
Total		41(63%)	24(37%)	65(100%)

Table-01 shows age and gender wise distribution of cases under study. Of the 65 cases, 41(63%) were male and 24(37%) were female. The age of the children were further categorized into three groups as follows, 0-4, 5-8 and 9-12 years. The most common age group that participated in this study was between

5 and 8 years constituting about 43% .More number of male patients were seen in all age groups.

However in comparison to the total male and female cases included under the study, there was no significance (p >0.05) observed in relation to sex and age in the groups.

Figure 1 Age and Gender wise distribution of cases under study

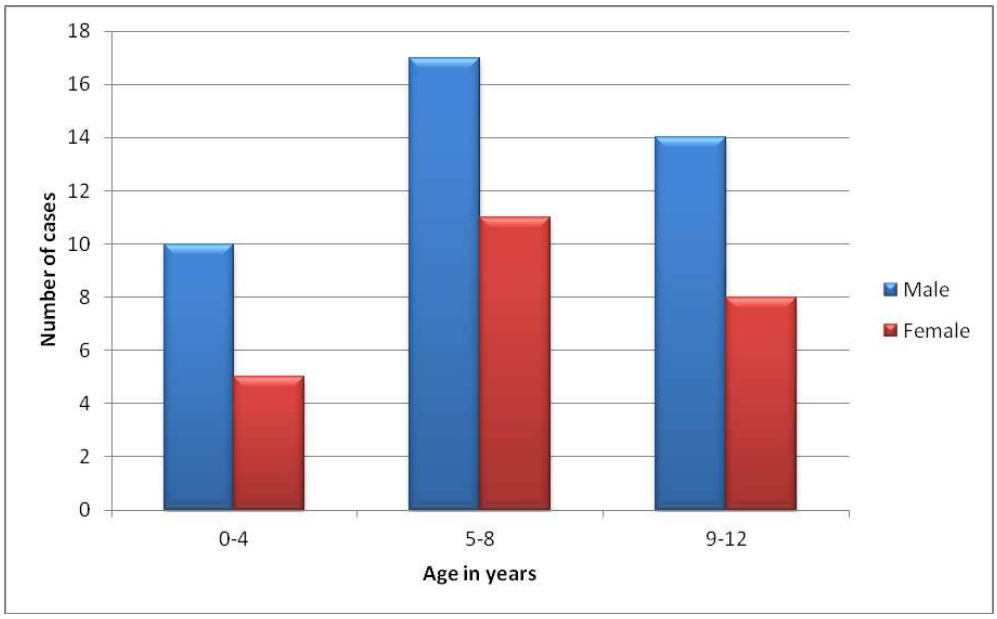
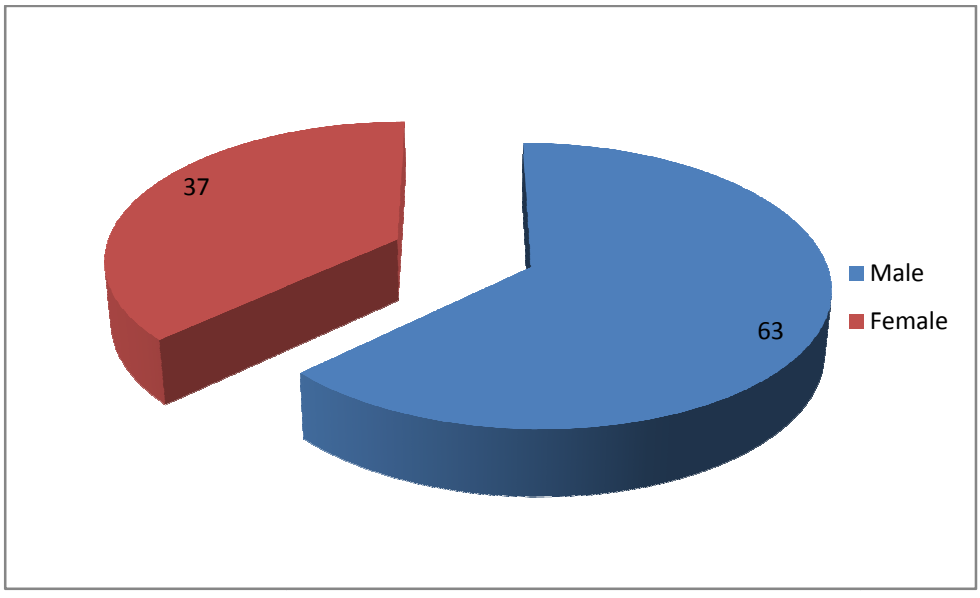


Figure 2 Gender wise distribution of cases under study (in percentage)



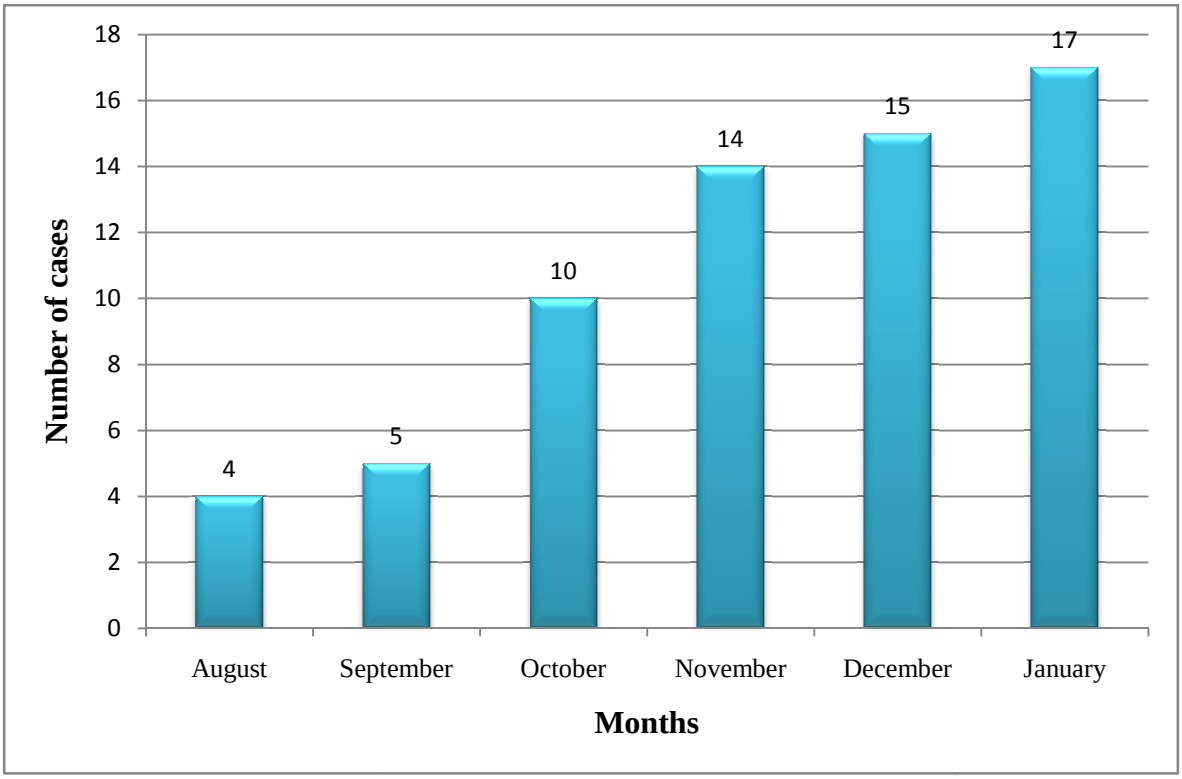
5.3. Monthwise distribution of admission of study group

Table 2 Month wise distribution of cases

Sl.No	Month of admission	Number of cases	Percentage
1	August	4	6.2%
2	September	5	7.7%
3	October	10	15.4%
4	November	14	21.5%
5	December	15	23.1%
6	January	17	26.1%

The above table shows the month wise distribution of cases admitted in the hospital during the study period. There was an increase in the number of cases during October (15.4%) compared to the previous two months. The cases then gradually increased and was on the rise during November (21.5%), December (23.1%) and January (26.1%). This corresponds to the monsoon season of this place.

Figure 3 Month wise distribution of cases under study



5.4 Symptoms of the study group

Table-03 and Fig-04 represents the clinical symptoms of the study population.

Table 3 Symptoms of the cases under study (n=65)

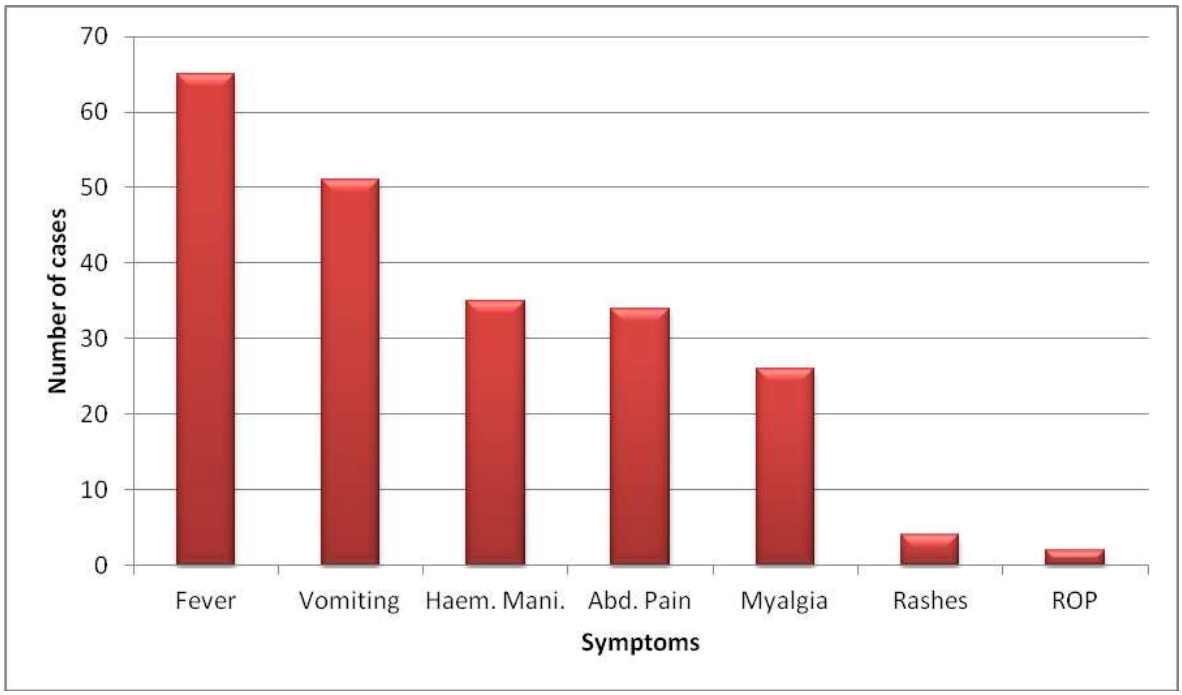
S.No.	Symptoms	Number of cases	Percentage
1	Fever	65	100
2	Vomiting	51	79
3	Haemorrhagic manifestations	35	54
4	Abdominal pain	34	52
5	Myalgia	26	40
6	Rashes	4	6
7	Retro orbital pain	2	3

Next to the predominant symptom of fever (100%), vomiting was seen in 51(79%) cases. Haemorrhagic manifestations like petechiae, bleeding gums, haemetemesis, malena, haematuria and subconjunctival haemorrhage were seen in 35(54%) cases.

Abdominal pain which may be due to gastrointestinal bleeding, liver injury and hepatomegaly in dengue were observed in 34(52%) cases. Myalgia was observed in 26(40%) cases.

Rashes were noted in 4(6%) cases. Retro orbital pain which is generally considered as a cardinal feature of dengue fever was noted only in 2(3%) cases in this study.

Figure 04 Symptom wise distribution of cases under study



5.5. Lab findings of the study group

Table 4 Laboratory findings of the cases under study (n=65)

S.no	**Investigations**	**Findings**	**No. of cases (%)**
1	**Haemoglobin**	< 10 gm/dl	10(18%)
		>10 gm/dl	55(82%)
2	**WBC (/cumm)**	<4000 cells	10(18%)
		>4000 cells	55(82%)
3	**HCT**	<35	17(26%)
		>35	48(74%)
4	**Platelets (/cumm)**	<50000	31(48%)
		>50000	34(52%)
5	**LFT**	Elevated	10(18%)
6	**Chest X ray**	Pleural effusion	3(5%)
7	**Ultrasonagram**	Ascites	7(11%)
		Hepatomegaly	6(9%)
		Gall bladder wall thickening	8(12%)
		Pleural effusion	16(25%)

Various lab investigations like Haemoglobin (Hb), WBC, Hematocrit (HCT), Platelet counts (PLT) and Liver function tests (LFT) were analysed.

Chest Xray (CXR) was evaluated for pleural effusion. Ultrasonogram (USG) was performed to look for ascites, hepatomegaly, and gall bladder wall thickening suggestive of acalculous cholecystitis and pleural effusion.

10(18%) cases had Hb less than 10 gm/dl and 55(82%) had more than 10 gm / dl. 55(82%) cases had a WBC count of more than 4000 cells and 10(18%) had less than 4000 cells. HCT was more than 35 in 48(74%) cases and less than 35 in 17(26%) cases.

PLT was less than 50,000 in 31(48%) cases and more than 50000 in 34(52%) cases. None of the patients had platelet count of more than 1,00,000 indicating that all had thrombocytopenia. In LFT, enzymes were found elevated in 10(18%) cases.

Pleural effusion was seen by CXR in 3(5%) cases and in 16(25%) cases by using ultrasound. Hepatomegaly was seen in 6(8%) cases and gall baldder thickening suggestive of acalculous cholecystitis was seen in 8(12%) cases in USG analysis.

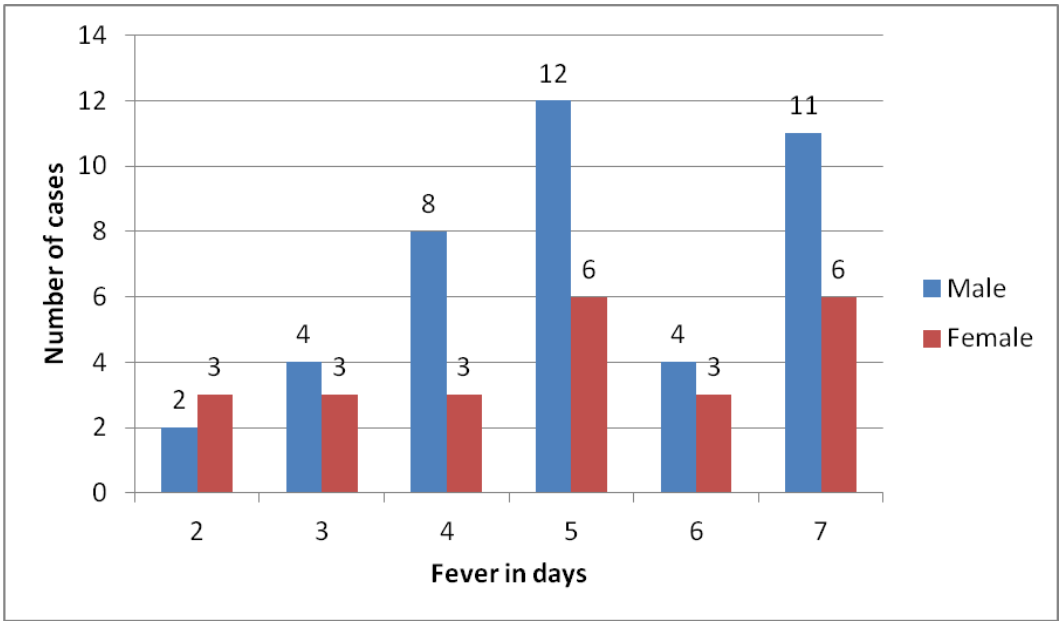
5.6 Day of fever on admission

Table 5 Fever in days of the study population (n=65)

Fever in days	No. of cases		Total
	Male	Female	
2	2	3	5(8%)
3	4	3	7(11%)
4	8	3	11(17%)
5	12	6	18(28%)
6	4	3	7(11%)
7	11	6	17(26%)
Total	39	26	65

Table-05 shows the day of fever, on which the patients were admitted and samples were collected for dengue investigations. 41(64%) cases were admitted within 5 days of onset of fever of which 26(40%) cases were males. Around 50% of cases were admitted between 3 to 5 days. After 5 days only 24 (37%) cases were admitted of which 11 were male and 6 were females.

Figure 5 Fever in days of the study population



5.7 NS1, IgM ELISA Test Results

All the 65 samples collected during the acute phase of fever were tested for NS1 antigen and IgM antibody by ELISA.

Table 6 Test Results of acute phase samples

Total samples tested	IgM/NS1/Both Positive	Negative
65	46	19

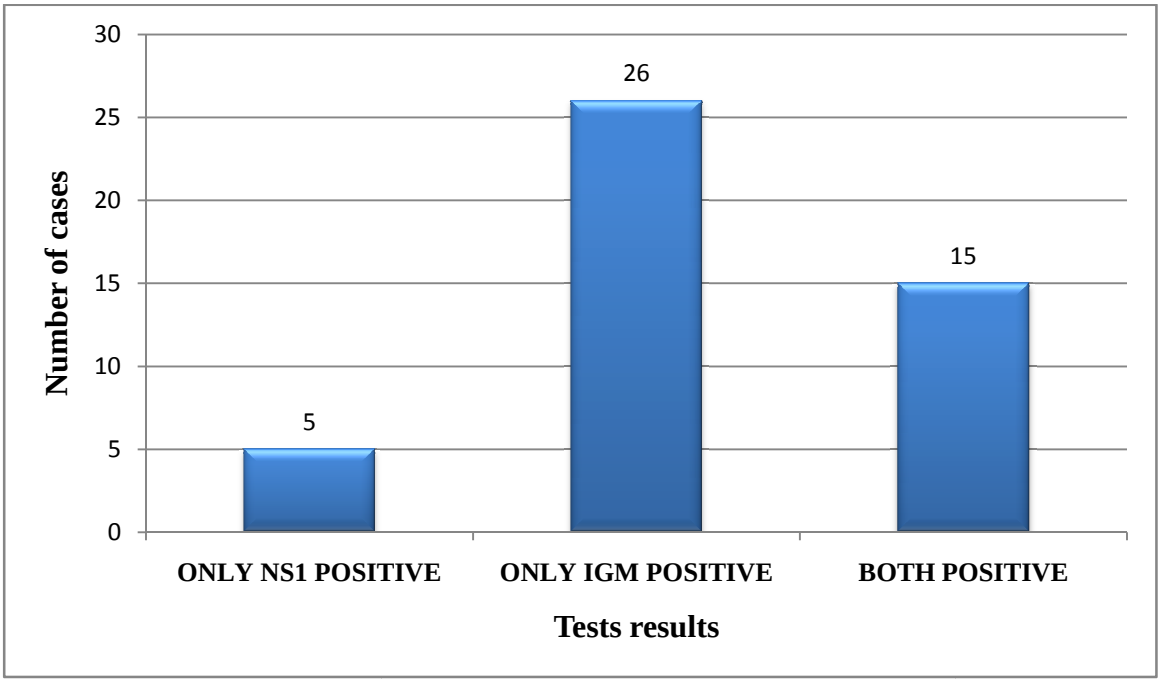
Of the 65 samples , 46(71%) tested positive for NS1, IgM or both. 19 (29.2%) samples tested negative for both the tests in the acute phase sera.

Table 7. NS1 / IgM ELISA results (n=65)

Result	Positive
Only NS1 positive	5
Only IgM positive	26
Both positive	15
Total positive	46

Only NS1 antigen was positive in 5 samples, only IgM was positive in 26 samples and both were positive in 15 samples.

Figure 6 Tests Results – NS1/IgM (n=65)



5.8. Fever Correlation with Test results

Table 8 Correlation of fever and results of NS1 and IgM among study population (n=65)

Fever in days	No. of samples tested	NS1 Positive		NS1 Negative		Percentage of NS1 Positive	Percentage of IgM Positive
		IgM Pos	IgM Neg	IgM Pos	IgM Neg		
2	5	0	0	3	2	0	60
3	7	2	2	1	2	57	43
4	11	4	0	6	1	27	91
5	18	3	2	7	6	28	56
6	7	2	0	3	2	29	71
7	17	4	1	6	6	29	59
TOTAL	65	15	5	26	19	31	63

Table – 08 correlates the day of fever and the test results among study population.

Of the total 65 samples that were collected and tested for NS1 and IgM, 20 were positive for NS1, 41 for IgM, 15 for both and 19 samples tested negative for both. Only NS1 was positive in 5 samples and only IgM was positive in 26 samples.

Of the 5 samples collected on day two of fever, 3 were positive for IgM, none was positive for NS1 and 2 were negative for both.

Of the 7 samples collected on day three of fever, 4 were positive for NS1, 3 were positive for IgM, 2 were positive for both and 2 were negative for both tests.

Of the 11 samples collected on day four, 4 tested positive for NS1, 10 for IgM, 4 were positive for both and 1 was negative for both NS1 and IgM.

Maximum number of samples was obtained on day five of fever. Of the 18 samples collected, 5 were positive for NS1and 10 for IgM. 3 samples tested positive for both and 6 were negative for both tests.

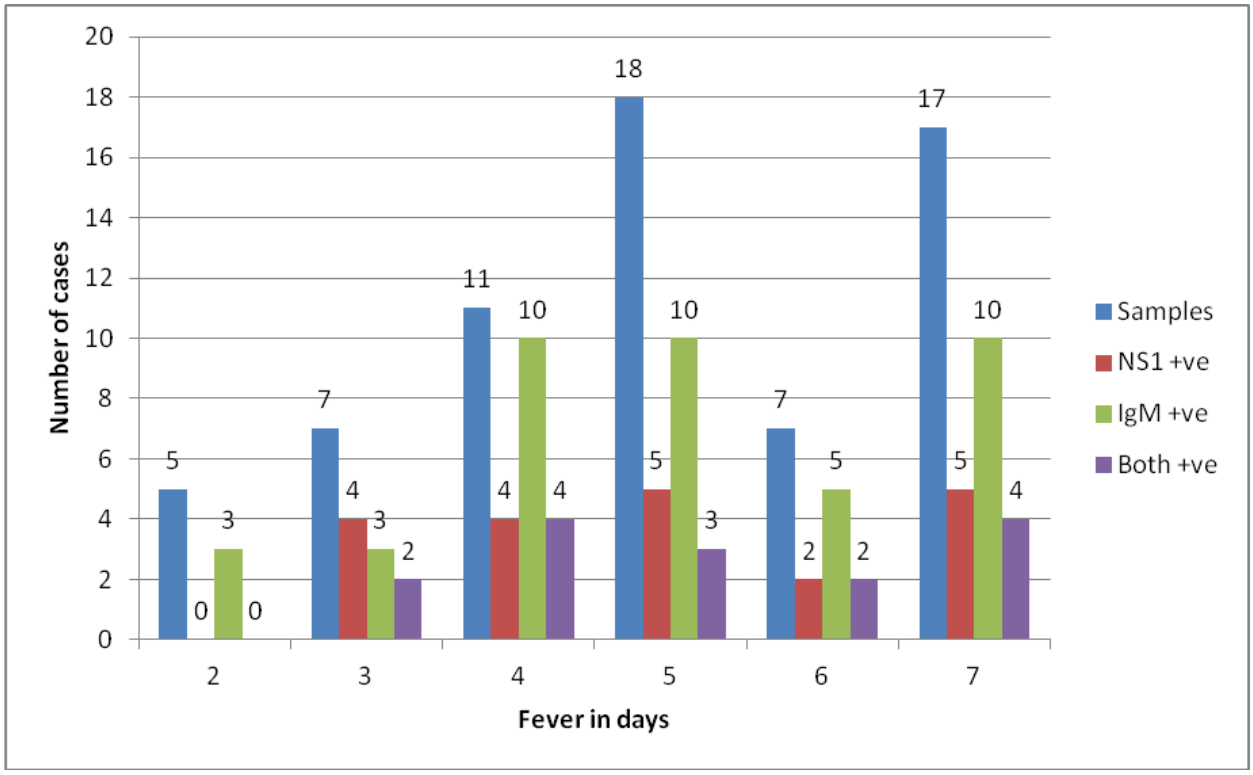
On day six of fever, 7 samples were obtained. Of which, 2 were positive for NS1 and 5 for IgM. 2 samples tested positive for both and 2 were negative for both.

Of the 17 samples collected on day seven of fever 5 was positive for NS1 and 10 for IgM. 4 were positive for both NS1 and IgM and 6 were negative for both.

More number of IgM positive cases were observed from day four onwards. Out of the 41 IgM positive cases, 35 had more than four days of fever.

Statistical analysis done by binary logistic regression method showed that NS1 detection in acute phase is not significant ($p > 0.05$) and IgM detection is significant **($p < 0.05$)**

Figure 7 Correlation of fever and results of NS1 and IgM among study population.



5.9 Significance of Testing Paired Sera

A second sample could be collected from 45 patients for whom IgM and IgG ELISA was performed. For the same 45 patients, IgG ELISA was performed for the acute phase samples which had been collected earlier.

Of the 45 samples, 12 had tested negative for NS1/IgM/IgG in the first sample. The following table analyses the results of the 12 negative samples.

Table 9 Analysis of paired sera for the initially negative samples (n=12)

Sl.No.	Sample 1 (Acute sera)		Sample 2 (Convalescent sera)		Dengue result
	IgM	IgG	IgM	IgG	
1	Neg	Neg	Neg	Neg	**Negative**
2	Neg	Neg	Neg	Neg	**Negative**
3	Neg	Neg	Neg	Neg	**Negative**
4	Neg	Neg	Neg	Neg	**Negative**
5	Neg	Neg	POS	Neg	**Seroconversion Positive**
6	Neg	Neg	POS	Neg	**Seroconversion Positive**
7	Neg	Neg	POS	Neg	**Seroconversion Positive**
8	Neg	Neg	Neg	POS	**Positive**
9	Neg	Neg	Neg	POS	**Positive**
10	Neg	Neg	Neg	POS	**Positive**
11	Neg	Neg	Neg	POS	**Positive**
12	Neg	Neg	Neg	POS (Low titre)	**Past infection**

Out of the 12 samples which were initially negative for IgM and NS1, 4 cases gave negative results for NS1, IgM, and IgG in the second sample also. So they were declared dengue negative cases.

Of the remaining 8 cases, three were positive for IgM (seroconversion) and were now declared as dengue confirmed cases.

Four cases were positive for IgG in the second sample and they were declared as secondary infection cases.

The remaining one case showed low IgG titre in comparison to the first IgG value and was declared as past infection with dengue.

Significance of using paired sera was analyzed by testing the 12 samples which were negative initially. The results are shown in Table - 09. Paired serum analysis is found to be statistically significant **($p<0.05$)** in the diagnosis of dengue infection.

5.10 Primary and Secondary dengue infection

The criteria used for classifying Primary and Secondary dengue infection were as follows. Primary infection was defined as an IgM positive/IgG negative in the first specimen and an IgM negative/IgG negative in the first specimen becoming IgM-positive/IgG-positive in the second specimen.

Secondary infection was defined as an IgM-negative/IgG-positive first specimen and an IgM-positive/ IgG-positive second specimen.

Table-10 gives the OD values of IgM and IgG in the acute and convalescent sera of the 45 samples with which they were classified into primary and secondary dengue infection.

Table – 10. Analysis of acute and convalescent phase sera (n=45)

Sl. No.	Sample 1 N^0	Sample 1 (OD value)		Sample 2 N^0	Sample 2 (OD value)		Type of Infection
		IgM cutoff value 0.488	IgG Cutoff value 0.247		IgM cutoff value 0.488	IgG cutoff value 0.247	
1	5	0.18	0.06	16	1.167	0.855	Primary
2	4	2.4	2.36	16	2.428	2.414	Secondary
3	4	2.42	2.4	18	0.504	2.365	Secondary
4	7	0.277	0.202	18	0.243	0.277	Negative
5	5	1.263	0.486	19	0.46	0.365	Primary
6	7	0.51	0.814	19	2.167	2.412	Secondary
7	6	2.43	2.419	18	2.423	2.425	Secondary
8	2	2.48	2.46	15	2.366	2.499	Secondary
9	7	2.557	2.489	18	2.495	2.426	Secondary
10	2	2.055	0.237	13	0.27	0.279	Primary
11	6	0.285	0.113	21	0.381	0.811	Secondary
12	5	2.446	0.046	16	2.369	0.871	Primary
13	5	0.146	0.164	16	0.224	0.407	Negative
14	7	0.753	1.524	17	1.411	1.763	Secondary
15	5	2.441	0.795	16	2.432	2.353	Secondary
16	5	2.502	0.526	15	2.502	0.336	Primary
17	4	2.566	0.091	18	2.447	0.496	Primary
18	7	2.424	0.089	16	2.436	0.501	Primary
19	7	0.373	0.026	22	0.224	0.33	Negative
20	5	2.436	1.193	22	1.826	1.521	Secondary
21	3	0.637	0.206	16	1.327	0.029	Primary
22	4	2.3	0.147	16	2.471	1.337	Primary
23	3	2.134	2.401	17	1.135	2.271	Secondary
24	7	2.498	2.469	18	0.34	0.169	Secondary
25	5	1.011	0.315	18	0.386	0.153	Primary
26	7	2.346	1.497	18	1.426	2.55	Secondary
27	5	0.2	0.783	18	0.31	0.719	Secondary
28	3	1.293	0.146	18	2.54	0.308	Primary
29	7	0.371	0.416	15	0.232	0.022	Negative
30	2	0.267	0.051	17	2.441	2.382	Primary
31	7	0.332	0.067	17	1.849	0.276	Primary
32	5	2.482	2.497	19	2.4	2.493	Secondary
33	5	2.519	2.606	18	2.364	2.344	Secondary
34	6	2.363	2.364	18	2.42	2.415	Secondary
35	6	0.382	1.073	12	0.196	0.066	Secondary
36	5	0.244	0.045	16	0.524	0.594	Past infection
37	7	2.524	2.515	15	2.407	0.341	Secondary
38	6	2.306	2.44	21	2.423	2.416	Secondary
39	6	2.437	0.044	22	2.449	0.202	Primary
40	5	1.201	0.119	12	2.519	0.196	Primary
41	4	2.576	0.138	17	0.316	0.126	Primary
42	5	2.406	2.376	17	2.364	2.385	Secondary
43	7	2.472	2.262	20	2.456	2.087	Secondary
44	6	2.463	0.056	17	2.534	0.54	Primary
45	4	2.466	2.476	16	2.067	2.349	Secondary

N^0 = Number of days from the start of the symptoms.

The following table consolidates the primary and secondary dengue cases.

Table 11 Primary and Secondary dengue infection

Total samples	Primary Dengue	Secondary Dengue
45	17	23

Of the 45 samples, 17 were declared as primary Dengue and 23 were declared as secondary Dengue infection

5.10.1 NS1 in Primary and Secondary dengue infection

Table – 12 . Correlation of NS1 with primary and secondary infection

Type of infection	Total cases	NS1		p value
		Positive	Negative	
Primary	17	10	7	0.013(<0.05)
Secondary	23	5	19	

Out of 17 lab confirmed primary dengue cases, NS1 was positive for 10 (59%) cases and out of the 23 secondary cases it was positive in only 5(22%) cases. This shows that NS1 is more sensitive in finding primary cases **(p <0.05)**.

5.11.2 Clinical findings in Primary and Secondary dengue infection

Table-13 analyses various factors like age, sex, haematocrit, platelet count, WBC count and USG findings in relation to primary and secondary dengue infections.

Table – 13. Clinical manifestations of primary and secondary infections

CHARACTERS			PRIMARY INFECTION	SECONDARY INFECTI0N	p value
Age in years	0 – 4	Male	4	3	NA
		Female	1	2	NA
	5 – 8	Male	3	8	NA
		Female	2	6	NA
	9 – 12	Male	4	3	NA
		Female	3	2	NA
Sex	Male		11/25 (44%)	14/25 (56%)	>0.05
	Female		6/16 (38%)	10/16 (62%)	
Platelet count	< 50000/cu.mm		7/21 (41%)	14/21 (58%)	**<0.05**
	>50000/cu.mm		10/20 (59%)	10/20 (52%)	
Haemorrhagic manifestations			8/22 (47%)	14/22 (58%)	>0.05
Hct	>35		12/33 (36%)	21/33 (64%)	**<0.05**
WBC	<4000/cu.mm		2/5 (40%)	3/5 (60%)	>0.05
USG	Pleural effusion		3/13 (23%)	10/13 (77%)	**<0.05**

Out of 40 cases, 19(46%) were in the age group of 5 to 8. In this group 14(74%) had secondary dengue infection. More number of males was seen in both primary and secondary infection compared to females. But statistical analysis using Fisher exact test showed that the difference was not significant ($p>0.05$).

21(51%) cases had a platelet count of less than 50000 cells. Of which 14(58%) cases were seen in secondary infection which is statistically significant **($p <0.05$).**

Haemorrhagic manifestations were more common in secondary infection but was not statistically significant ($p>0.05$).

21 out of the 33(64%) cases had a haematocrit of more than 35 in secondary infection. Analysis by paired t test showed this to be significant **($p<0.05$).**

Three out of the 5(60%) secondary dengue cases had WBC of less than 4000. More cases had pleural effusion in secondary infection and was statistically significant **($p<0.05$).**

6. DISCUSSION

Dengue is an emerging public health problem in India. In the present study 65 children, who were admitted in the paediatric ward of Tirunelveli Medical College with a clinical suspicion of dengue virus infection were analyzed.

6.1 Age and Gender

Amongst the fever cases admitted, 65 children fulfilled the inclusion criteria and were included in the study. Majority of the cases were between 5-8 years. Similar finding was reported by Nisalak et al[30] (5-9yrs) and Kabra et al[33] (8 years).

In the present study the male to female ratio was 1.7:1. Similar pattern was seen in the analysis of 2006 dengue outbreak in North India by Chandrakanta et al[74] (1.6:1) and Mittal et al[75](1.3:1). Male children were more commonly affected than female children in the studies of Neeraja et al [19] (2:1) and Aggarwal et al[36] (3:2) whereas Maria Guzman et al [2](1:1.4), Kabra et al[33] and Ole Wichman et al[32] (0.96:1) reported in their studies that female children were slightly more affected than male children. But overall, a review of reported literature shows no sex predilection for the disease Statistical analysis revealed that there was no association between age and gender in the present study.

6.2 Seasonal Distribution

The study was done from August 2011 to January 2012. Analysis of the data was done for each month to identify the seasonal variance of dengue infection. A gradual increase in the occurrence of cases was seen from September with a peak in December and January which corresponds to the monsoon rainfall of this region. This results in stagnation of water, which facilitates vector breeding. Between November and January there were 46 (70.7%) cases enrolled in this study. Similar studies indicating the correlation between emergence of dengue and monsoon was reported in South India by S.C. Tewari et al[6] and Singh J et al[28] in Central India by PM Ukey et al, [18] in Karnataka by Aswini kumar et al[76] and in Karachi by Khan et al.[77] This finding indicates that preventive measures play an important role during water stagnation periods, in the fight against dengue infection.

6.3 Clinical Symptoms of the cases under study

The most common presenting symptom in this study was fever (100%) which was the essential criteria for inclusion in the study. Various studies done in India by Aggarwal et al,[36] Chandrakanta et al,[74] Mittal et al[75] and have stated fever as the commonest presenting symptom.

Vomiting which is a warning sign of severe infection was seen in 51(79%) cases. It was observed in 83% of cases by Narayanan et al,[34] Retgeri et al[38] and 82% of cases similar to the present study. Aggarwal et al[36] has noted lesser incidence of 68% in his study.

In the present study abdominal pain was seen in 34 (52%) cases similar to Batra et al[78] (52%). Abdominal pain in 61% of dengue infected cases was observed in a study done by Ratgeri et al.[38] Aggarwal et al[36] and Narayanan et al[34] observed abdominal symptoms of 49% & 23% respectively in their studies.

Khan et al[77] has reported that abdominal pain could be due to liver injury, gastro intestinal bleeding and acalculous cholecystitis or capsulitis caused by the dengue virus. It is important to remember that infections that cause fever and gastro intestinal symptoms like enteric fever, leptospirosis, enteroviral infections are endemic in India and may often lead to a delay in dengue diagnosis. Hence it is suggested that all forms of dengue fever should be included in the differential diagnosis of patients with fever and gastrointestinal symptoms.

Next common clinical symptom was haemorrhagic manifestations like epistaxis, bleeding gums, haematemesis and malena due to plasma leakage and increased vascular permeability which was seen in 35(54%) cases in the present study. Haemorrhagic manifestation was noticed in more than 30% of the cases by Aggarwal et al,[36] in 40% by Ratgeri et al[38] and in 66.1% by Narayanan et al.[34]

Maculopapular rash, which is an important finding in dengue infection, was seen in only 6% of cases in this study. Various authors have reported skin rashes ranging from 22% to 60% in their studies. 37.5% children in a study by Chandrakanta et al[74] had skin rash, while 24.4% was observed by Batra et al[78]

and 26% by Mittal et al.[75] The low incidence in this study may be due to difficulty in identifying the rash in dark skin complexion seen in this part of the country.

Retro orbital pain which is included in the clinical diagnosis of dengue infection by WHO, was seen in only 3% of cases. Neeraja et al[19] (7%) and Narayanan et al[34] have reported an incidence of 12% of cases having retro orbital pain in their study. Since a good number of patients were in the age group of less than 8 years in the present study, it was difficult to elicit the history.

6.4 Lab Investigations

Various lab investigations like Haemoglobin (Hb), WBC, Hematocrit (HCT), Platelet count (PLT), Liver function tests (LFT) and Imaging techniques like Chest Xray and USG reports were analyzed.

Haemoglobin and haematocrit which was collected at the time of admission showed that 78% of the cases had haematocrit of more than 35. This value is taken as a haemoconcentrated state as per Balasubramanium et al[80] defining the severity of the problem. Haemoconcentration has been reported in the studies of Aggarwal et al[36] (42%) and Narayanan et al[34] (24.1%). Patients with haemoconcentration were monitored with serial haematocrit taken once in 2 hours, according to WHO guidelines and were aggressively managed.

A platelet count of less than $1x10^5$ cells indicating thrombocytopenia is one of the criteria in the clinical diagnosis of dengue fever. This is an important

and cost effective investigation in dengue suspected cases, where ready access to viral culture or identifying antigen, antibody markers are not available. In this study all patients had a platelet count of less than $1x10^5$ cells, of which 48% of cases had a count of less than $5x10^4$ cells. Low platelet counts were reported by Narayanan et al[34] (43%) and Aggarwal et al[36] (69%) in their studies.

Various studies have reported that leukopenia of less than $4x10^3$ is a significant finding in DHF and DSS (Kabilan et al[**39**]). In the present study total leucocyte count of less than $4x10^3$ was observed in 18% of cases. Similar results have been shown by Aggarwal et al[36] (15%) and Retgeri et al[38] (24%).

Features of plasma leakage like pleural effusion, ascites, hepatomegaly are cardinal features in DHF/DSS. In the present study pleural effusion was picked up by chest X-ray in 3(5%) cases and by ultrasonogram in 16(25%) cases. Similar results of USG being a superior diagnostic test for detecting pleural effusion has been reported by Balasubramanium et al[80] (23%).

Gall bladder wall thickening due to acalculus cholecystitis was noted in 12% of cases which was much lower than the study of Balasubramanian et al[80] (32%).

6.5 Test Results

The gold standard in confirming dengue is by viral culture. But it is a time consuming and technically demanding procedure which may not be available in many public health hospitals in developing countries. So antigen-antibody detection is the cornerstone in the diagnosis of early dengue infection.

Antigen detection by NS1 capture ELISA can be done as early as the first day to as late as eighteenth day of onset of symptoms (Xu et al [41]) with sensitivity as good as RT-PCR.

In the present study, out of the 65 suspected dengue cases, 45 (70.8%) tested positive for NS1 or IgM or both. 19 (29.2%) samples tested negative for both the tests in the acute phase sera.

Of the 65 samples tested, NS1 was positive in 20(31%) cases. Many authors like Ivani Bisardi et al[50] (99.3%), Chua et al[48] (91.6%), Laurent Thomas et al[49] (67.1%) have stated that the incidence of detecting NS1 antigen is more sensitive in acute phase samples.

In the present study more NS1 positive cases were seen in day 5 and 7 with five cases on each day.

Mini P Singh et al[43] considered NS1 testing to be useful till 3rd day of illness and Dussart et al[42] observed NS1 to be useful till 4 days of the illness in the diagnosis of dengue infection. Both the authors did not find it to be useful beyond the 5th day of illness.

Alcon et al[40] found that NS1 can be detected upto 10 days, with higher sensitivity on day 4 & 5. Xu et al[41] found that NS1 can be found up to 18 days with peak at days 6-10.

However in the present study the sensitivity of detection of NS1 antigen was found to be low, in first 5 days of illness. Studies done by many authors have reported the incidence of detecting NS1 antigen in the acute phase

samples. Shrivastava et al[45](16%), Koraka et al[72] (18%), Kulkarni et al[79](30%), Remirez et al[81](30%), Kassim et al[56] (32.2%) and Anita Chakravarthi et al[62](39.7%)]. The lower detection rate may be due to low viremic index, early immune complex formation or due to the serotypes DENV 2 & 4 which was not confirmed in the study.

IgM antibody appears as early as 3 days and reaches a peak in 2 weeks and remains positive for 2 to 3 months. In the present study, 41(63%) cases tested positive for IgM antibody. More number of IgM positive cases were seen from day 4 onwards compared to NS1 antigen. Testing of IgM after 4 days was found to be significant in the present study. Similar reports by many other studies like Sang et al[52] (3days) and Innis et al [53](4 days) implies the significance of IgM antibody testing after day 3 in diagnosis of acute dengue infection.

From this study we could assess that NS1 was sensitive in diagnosing 31% of cases and this increased to 71%, by a margin of 40% when combined with IgM antibody test in diagnosing dengue between days 2 and 7.

Substantial increase in sensitivity was obtained by Duong et al[47] (57.5% to 85%), Kassim et al[56] (51% to 62%), Stuart D.Blackshell et al[57] (45 to 57%) and Kulkarni et al[81](30 to 40%) in their studies implying that antigen and antibody testing should be combined in the early diagnosis of dengue infection.

6.6 Significance of testing paired sera

Significance of collecting paired sera was analyzed by testing 12 samples which were negative initially. Three samples which were initially IgM negative seroconverted and were IgM positive in the convalescent sample. Four samples were positive for IgG which were considered secondary infection cases.

If paired sera had not been collected and analyzed, these 7 cases would have been missed and reported as dengue negative cases. Paired serum analysis in this study was found to be statistically significant. WHO insists that testing paired sera is mandatory for confirming dengue infections.

6.7 Primary and secondary infections

Primary infection is defined by positive IgM and negative IgG, secondary infection is by both IgM and IgG positive in the serum samples. With these criteria primary and secondary infections could be clearly delineated.

In the present study 17 (38%) cases were primary infections and 23(52%) cases were secondary infections. Kuno et al[60] in their study also observed 41% and 52% of primary and secondary dengue infections respectively. Neeraja et al[19] and Ole Wichman et al have however observed more secondary dengue cases of 73% & 78% respectively.

Small children with dengue infections have mostly primary infection and are likely to develop only mild symptoms & would not have required hospitalization. Being an endemic area the study group had more risk of acquiring infection by different serotypes leading to more number of secondary

dengue infections. These may be the reasons why more secondary dengue infections have been reported in the present study.

DHF/DSS must be considered a children's disease because the paediatric age group are innately at increased risk compared with adults. Due to the high mortality in DHF/DSS cases accurate diagnosis is most essential.

The demographic profile and laboratory investigations of the primary and secondary dengue cases were analyzed. The clinical presentation of the children with primary dengue infections was less severe in the present study. Age and gender between primary and secondary infections was not statistically significant.

Platelet count $<10^5$ was noted in 41% of primary infections and 58% of secondary infections which was statistically significant. DHF/DSS are more common in secondary dengue infections. Testing of platelet count is important and planning the management accordingly is vital in dengue cases.

Although haemorrhagic manifestations like epistaxis, haematemesis and malena were noted more in secondary dengue infections (58% vs 47%) it was not found to be statistically significant in the present study.

Haematocrit value in secondary dengue infections (64%) was statistically significant in the present study. Balasubramanian et al[80] reported that 57.4% cases showed haemoconcentration in secondary dengue infections. Neeraja et al[19] reported 40% in their study.

In the present study USG findings of plasma leakage (77%) amongst secondary dengue infections was found to be statistically significant similar to the study of Balasubramanian et al[80](88.57%).

6.8 NS1 and its Correlation to Primary and Secondary dengue infections

The results of NS1 in primary and secondary infection cases were analyzed. In the 17 primary dengue infection cases, 59% were NS1 positive, whereas amongst the 23 secondary dengue infection cases, NS1 positivity was observed only in 22% implying that NS1 detection is more sensitive in primary dengue rather than secondary dengue infections. Duong et al[47], Kumarasamy et al[69], Sekaran et al[70] and Koraka et al[72] in their studies also observed that NS1 is positive in primary than secondary infection.

Although NS1 antigen is a highly specific and extremely reliable marker for the diagnosis of dengue infections, low positivity in secondary infections as noted by Kwoon Yong Pok et al[46] and Young et al may be due to low viremia, immune complex formation and virus serotype. As the present study had more secondary infection it would have been reason for the low detection rate in acute phase samples. From the present study, it is inferred that serological tests to detect dengue viral antigen and antibodies in the serum samples had greatly enhanced the ability to effectively & efficiently diagnose dengue infection.

7. SUMMARY

The present study aimed at analysing the role of NS1 antigen detection during the acute phase and its correlation in primary and secondary dengue infections.

- 65 cases of suspected dengue infections in the age group of 0-12 years were included in the study.
- Majority of the cases were between 5-8 years (43%).
- More number of male cases were noted (63%).
- Increased number of cases was seen during the months of November to January (70.7%).
- Fever (100%), vomiting (79%), haemorrhagic manifestations (54%), abdominal pain (52%), rashes (6%) and retro orbital pain (3%) were the symptoms noted.
- Haematocrit >35 was noted in 78% of cases.
- All the patients had a platelet count of $<1x10^5$ cells. 48% of the cases had count of $<5x10^4$ cells.
- Total leucocyte count of <4000 was observed in 18% of the cases.
- Pleural effusion was picked up by chest X-ray in 5% cases and by ultrasonogram in 25% cases.

- NS1 antigen and IgM antibody testing was done to analyse their role in the diagnosis of acute dengue infections. Out of the 65 suspected cases, 46 samples gave a positive result for either NS1 or IgM in the acute phase sera, the seropositivity being 70.8%.
- NS1 was positive in 31% cases and the sensitivity of detection of NS1 antigen was found to be low, in first 5 days of illness.
- More NS1 positive cases were seen in day 5 and 7 with five cases on each day.
- 63% tested positive for IgM antibody and more number of IgM positive cases were seen from day 4 onwards compared to NS1 antigen.
- NS1 was sensitive in diagnosing 31% of cases and this increased to 71%, by a margin of 40% when combined with IgM antibody testing.
- A paired serum was collected from 45 patients for whom analysis of IgM & IgG was done to differentiate primary and secondary dengue infections.
- Three samples which were initially IgM negative, seroconverted and were IgM positive in the convalescent sample.4 samples were positive for IgG which were considered secondary infection cases. If paired sera had not been collected and analyzed, these 7 cases would have been missed and reported as dengue negative cases.
- In the present study 17 cases were primary infections and 23 cases were secondary infections. More number of secondary infections were noted.

- Platelet count $<10^5$ was noted in 41% of primary infections and 58% of secondary infections.
- Haemorrhagic manifestations like epistaxis, haematemesis and malena were noted more in secondary dengue infections (58%).
- Haematocrit value more than 35 was more in secondary dengue infections (64%).
- An USG finding of plasma leakage was 77% amongst secondary dengue infections.
- NS1 was positive in 59% of primary cases and only in 22% of the secondary cases. NS1 detection was more sensitive in primary dengue rather than secondary dengue infections.The low positivity of NS1 antigen in the acute samples would have been due to increased number of secondary dengue infections seen in the present study.

8. CONCLUSION

- This study highlights the combined use of NS1 antigen and IgM antibody in the diagnosis of dengue infection in the acute phase.
- Testing of paired sera is essential in diagnosis of dengue infection and differentiating primary and secondary dengue infection.
- More NS1 positive cases were seen in days 5 and 7 only. It was low in the first five days of illness.
- IgM was found to be more positive than NS1 in samples collected after the fifth day of illness.
- More number of secondary dengue infections was noted in the study.
- High NS1 positivity was noted more in primary dengue infection than secondary dengue infection.
- The low positivity of NS1 would have been due to immune complex formation as more secondary cases were observed. It would have also been due to low viremia or due to the virus serotype which was not confirmed.

S.NO	AGE	SEX	FEVER	MYALGIA	VOMITI	ROP	RASH
1	9	M	5	YES	YES	NO	NO
2	12	M	4	NO	YES	YES	NO
3	10	M	4	NO	YES	NO	NO
4	12	F	7	YES	YES	NO	NO
5	10	M	5	NO	YES	NO	NO
6	6	M	6	YES	YES	NO	NO
7	7	F	4	NO	NO	NO	NO
8	8	M	2	NO	YES	NO	NO
9	4	M	7	NO	NO	NO	NO
10	12	F	2	YES	YES	NO	NO
11	6	F	8	YES	YES	NO	NO
12	3	M	5	NO	YES	NO	NO
13	12	M	5	YES	NO	NO	NO
14	6	M	7	NO	YES	NO	YES
15	5	M	7	YES	YES	NO	NO
16	4	M	5	NO	NO	YES	NO
17	2	F	4	NO	NO	NO	NO
18	9	M	7	NO	YES	NO	NO
19	5	M	7	NO	YES	NO	NO
20	2	F	5	NO	YES	NO	NO
46	5	F	3	NO	YES	NO	NO
22	4	F	6	NO	YES	NO	NO
23	8	F	3	NO	YES	NO	NO
24	3	M	7	NO	YES	NO	NO
25	9	F	5	NO	YES	NO	YES
26	8	M	7	NO	YES	NO	NO
27	12	F	5	YES	YES	NO	NO
28	8	M	1	YES	YES	NO	NO
29	8	M	7	YES	YES	NO	NO
30	7	F	2	YES	YES	NO	YES

31	11	F	9	YES	NO	NO	NO
32	3	M	5	NO	YES	NO	NO
33	5	M	5	NO	YES	NO	NO
34	12	M	4	YES	NO	NO	NO
35	7	F	6	NO	YES	NO	NO
36	2	F	5	NO	YES	NO	NO
37	7	F	7	YES	YES	NO	NO
38	6	M	6	YES	NO	NO	NO
39	7	M	7	YES	YES	NO	NO
40	8	M	5	YES	YES	NO	NO
41	10	M	3	YES	YES	NO	NO
42	5	M	5	NO	YES	NO	NO
43	6	F	7	NO	YES	NO	YES
44	3	M	6	YES	NO	NO	NO
45	9	F	4	YES	YES	NO	NO

MASTER CHART

BLEEDING	ABD PAIN	HB	WBC	HCT	PLT	CXR	USG	LFT	S1
YES	YES	12.2	7700	38,3	44000	NORMAL	NORMAL	N	5
YES	YES	13.1	5000	39.9	33000	NORMAL	ASCITIS	N	4
YES	YES	12.5	3400	39	60000	NORMAL	PF/AS/GB	N	4
NO	YES	12	5300	39	98000	NORMAL	NORMAL	N	7
NO	YES	12.8	3800	40.2	1.2	NORMAL	NORMAL	ELEVATED	5
YES	YES	12.2	2900	36.7	84000	NORMAL	GB	HIGH	7
YES	YES	18.1	8200	55.9	28000	NORMAL	NORMAL	HIGH ENZ	6
NO	NO	11.6	13,400	38.1	47000	NORMAL	PF	N	2
YES	NO	10.2	9100	37.5	86000	NORMAL	PF,GB,	N	7
NO	NO	13.1	10,100	41.5	98000	NORMAL	NORMAL	N	2
NO	YES	12.8	6500	36	1.62	NORMAL	NORMAL	N	6
NO	NO	10.3	2,700	34.6	1.4	NORMAL	NORMAL	N	5
YES	NO	13.7	2700	43.2	96000	NORMAL	NORMAL	N	5
NO	YES	8.8	17,700	38	23000	NORMAL	HEPA	N	7
YES	YES	13.2	8200	39.4	68000	NORMAL	PF,A	N	5
NO	YES	11.2	3,800	35	92000	NORMAL	HEPA	N	5
NO	NO	10.7	13,600	34.1	70000	NORMAL	NORMAL	N	4
NO	YES	14.9	5,200	45.5	68000	NORMAL	HEPA	N	7
NO	NO	10	11,000		90000	NORMAL	NORMAL	N	7
YES	NO	12.3	7,000	38.3	49000	NORMAL	NORMAL	N	5
YES	NO	12.2	6,500	39.4	96000	NORMAL	BLPF	N	3
YES	NO	10.7	6,200	35	81000	NORMAL	HEPAT	N	4
NO	YES	11	5,400	34.4	25000	NORMAL	NORMAL	N	3
NO	NO	9.5	12,400	30	58000	NORMAL	PF	N	7
YES	NO	12.2	5,800	38	85000	NORMAL	NORMAL	N	5
YES	YES	15..7	11,800	47.7	18000	PF	PF,AS	ELEVATED	7
NO	NO	13	34,000	39	72000	NORMAL	NORMAL	N	5
NO	NO	12	8,300	37.3	1.2	NORMAL	NORMAL	N	3
YES	NO	9.6	4,600	35.7	71000	NORMAL	NORMAL	N	7
NO	NO	9.5	4,400	37	43000	NORMAL	NORMAL	N	2

YES	YES	9.9	4,600	31	2.01	NORMAL	LIV,SPLE	N	6
NO	YES	10.1	5,400	31.8	47000	NORMAL	PF	ELEVATED	5
YES	YES	12.9	18,000	43	55000	NORMAL	NORMAL	N	5
YES	NO	14	8,200	49	44000	NORMAL	NORMAL	N	6
NO	YES	12.2	5,000	39	78000	NORMAL	NORMAL	N	6
YES	YES	10.2	6,200	40.2	68000	NORMAL	NORMAL	N	5
YES	NO	13	5,000	39.9	46000	NORMAL	GB	HIGH	7
YES	NO	11	4,600	38	90000	NORMAL	PF	N	6
NO	NO	13.2	5,100	41.7	88000	NORMAL	NORMAL	N	6
YES	NO	11.3	13,600	33.9	80000	NORMAL	NORMAL	ELEVATED	5
YES	YES	13.1	58,000	39.9	20000	PF	PF,GB,LIV	N	4
NO	NO	14.5	9,300	36	46000	NORMAL	PF	N	5
YES	YES	15	9,200	45.3	29000	NORMAL	RF,AS	N	7
YES	NO	9.7	10,900	33.1	73000	RF	RF,AS	N	6
NO	YES	14.5	4,500	46.2	21000	NORMAL	RF	N	4

NS1 OD	NS UNI	IgM OD	IgM UNI	IgG OD	IgG UNIT	S2	IgM OD	IgM UNIT	IgG OD	IgG UNIT
0.07	1.21	0.18	3.76	0.06	2.429	16	1.167	24.36	0.855	34.615
2.35	40.79	2.4	50.104	2.36	95.55	16	2.428	50.69	2.414	97.73
0.07	1.21	2.42	50.52	2.4	97.17	18	0.504	10.52	2.365	95.75
0.669	1.19	0.277	5.78	0.202	8.178	18	0.243	5.07	0.277	11.21
0.065	1.12	1.263	26.37	0.486	19.68	19	0.46	9.6	0.385	15.58
0.137	2.37	0.51	10.65	0.814	32.96	19	2.167	45.24	2.412	97.65
0.048	0.83	2.43	50.73	2.419	97.94	18	2.423	50.58	2.425	98.18
0.057	0.98	2.48	51.77	2.46	99.19	15	2.366	49.39	2.499	101.1
2.528	43.88	2.557	53.38	2.489	100.77	18	2.495	52.09	2.426	98.21
0.056	0.97	2.055	42.9	0.237	9.59	13	0.27	5.64	0.279	11.29
0.115	1.99	0.285	5.94	0.113	4.57	21	0.381	7.95	0.811	32.83
2.446	42.46	2.446	51.06	0.046	1.82	16	2.369	49.46	0.871	35.26
0.042	0.78	0.146	3.05	0.164	6.64	16	0.224	4.68	0.407	16.48
0.061	1.05	0.753	15.72	1.542	62.43	17	1.411	29.46	1.763	71.38
2.173	37.72	2.441	50.96	0.795	32.19	16	2.432	50.77	2.353	95.26
0.056	0.97	2.502	52.23	0.526	21.29	15	2.502	52.23	0.336	13.68
2.554	44.34	2.566	53.57	0.091	3.68	18	2.447	51.08	0.496	20.08
2.405	41.75	2.424	50.6	0.089	3.6	16	2.436	50.86	0.501	20.28
0.113	1.96	0.373	7.79	0.026	1.01	22	0.224	4.68	0.33	1.33
0.141	2.44	2.436	50.86	1.193	48.29	22	1.826	38.16	1.521	61.58
0.16	2.77	0.637	13.29	0.206	8.34	16	1.327	27.7	0.029	1.17
2.485	43.1	2.3	48.01	0.147	5.95	16	2.471	51.59	1.337	54.12
0.058	1	2.134	44.55	2.401	97.2	17	1.135	23.69	2.271	91.94
0.06	1.04	2.498	52.15	2.469	99.54	18	0.34	7.09	0.169	6.84
0.055	0.95	1.011	21.1	0.315	12.75	18	0.386	8.05	0.153	6.19
2.332	40.48	2.346	47.88	1.497	60.61	18	1.426	29.77	2.55	91.29
0.076	1.31	0.209	4.36	0.783	31.7	18	0.31	6.47	0.719	29.1
2.457	42.6	1.293	26.99	0.146	5.91	18	2.54	53.02	0.308	12.47
0.058	0.97	0.371	7.74	0.416	16.84	15	0.232	4.84	0.022	0.89
0.057	0.98	0.267	5.57	0.051	2.06	17	2.441	50.96	2.382	96.44

0.048	0.83	0.332	6.93	0.067	2.71	17	1.849	38.6	0.276	11.17
0.144	2.5	2.482	51.82	2.497	101.9	19	2.4	50.1	2.493	100.93
0.058	1	2.519	52.59	2.606	105.51	18	2.364	49.35	2.344	94.89
0.046	0.79	2.363	49.33	2.364	95.71	18	2.42	50.52	2.415	97.77
0.042	0	0.382	7.97	1.073	43.44	12	0.196	4.05	0.066	2.67
0.078	1.35	0.244	5.09	0.045	1.821	16	0.542	11.31	0.594	24.04
0.085	1.47	2.524	52.69	2.515	101.82	15	2.407	51.21	0.341	13.8
0.362	6.28	2.306	48.14	2.44	98.78	21	2.423	51.55	2.416	97.81
2.433	42.23	2.437	50.88	0.044	1.781	22	2.449	51.13	0.202	8.18
2.337	40.57	1.201	25.07	0.119	4.817	12	2.519	52.59	0.196	7.93
2.556	44.4	2.576	53.78	0.138	5.587	17	0.316	6.59	0.126	5.1
0.04	0.69	2.406	50.23	2.376	96.19	17	2.364	49.35	2.385	96.56
2.307	40.05	2.472	51.6	2.262	91.58	20	2.456	51.27	2.087	84.49
2.408	41.8	2.463	51.42	0.056	2.27	17	2.534	52.9	0.54	21.86
0.035	0.6	2.466	51.48	2.476	100.24	16	2.067	43.15	2.349	95.1

MASTER CHART

S.NO	AGE	SEX	FEVER	MYALGIA	VOMITI	ROP	RASH	BLEEDING	ABD PAIN	HB	WBC	HCT	PLT	CXR	USG	LFT	S1	NS1 OD	NS UNI	IgM OD	IgM UNI	IgG OD	IgG UNIT	S2	IgM OD	IgM UNIT	IgG OD	IgG UNIT
1	10	M	3	NO	YES	NO	NO	YES	YES	14	14000	36	27,000	NORMAL	PF/AS/GB	N	4	0.06	1.45	1.638	34.26							
2	5	M	3	NO	YES	NO	NO	YES	LIVER	11	6000	41.9	53000	NORMAL	PF/AS/GB	N	4	0.2	4.83	1.48	30.96							
3	4	M	7	NO	YES	NO	NO	NO	YES	14.5	6600	46.3	64000	NORMAL	NORMAL	ELEVATEDN	7	0.09	2.17	1.099	22.99							
4	8	M	2	YES	YES	NO	NO	NO	NO	12	10400	37.7	3.3	NORMAL	NORMAL	N	3	2.52	54.07	0.105	2.19							
5	4	M	5	NO	NO	NO	NO	YES	NO	11.6	3100	30.7	1.4	NORMAL	NORMAL	N	5	0.05	1.07	0.087	1.82							
6	12	M	4	NO	NO	NO	NO	NO	NO	13.1	3200	41.3	86000	NORMAL	NORMAL	ELEVATEDN	4	0.06	1.28	0.688	14.39							
7	3	M	4	NO	NO	NO	NO	YES	NO	7.9	9000	25.3	67000	NORMAL	NORMAL	N	4	0.113	2.42	1.22	25.52							
8	10	M	3	NO	NO	NO	NO	YES	YES	12.6	10,300	33.4	100000	NORMAL	NORMAL	N	3	0.11	2.36	0.092	1.92							
9	5	M	2	NO	YES	NO	NO	YES	YES	14.3	15,400	44.2	13000	NORMAL	GB	ELEVATEDN	3	0.093	1.99	0.077	1.61							
10	8	F	2	NO	YES	NO	NO	YES	YES	16.6	5700	53.8	42000	NORMAL	NORMAL	N	2	0.216	4.61	0.544	11.38							
11	3	F	4	NO	YES	NO	NO	NO	NO	12	4400	38.6	92000	NORMAL	NORMAL	N	5	2.469	52.98	0.04	0.83							
12	11	M	7	NO	YES	NO	NO	YES	YES	12.9	6300	40.8	58000	NORMAL	HEPATO	N	7	0.086	1.82	0.042	0.87							
13	7	F	5	YES	YES	NO	NO	NO	NO	10.9	3000	35	23000	NORMAL	NORMAL	N	5	1.492	32.01	0.091	1.9							
14	10	F	7	NO	YES	NO	NO	YES	YES	15.2	7800	47.4	20,000	NORMAL	NORMAL	N	7	2.447	52.51	0.021	0.43							
15	8	M	2	YES	NO	NO	NO	NO	NO	8.6	9800	31.4	1.2	NORMAL	NORMAL	N	2	0.127	2.72	0.091	1.9							
16	3	m	2	NO	YES	NO	NO	NO	NO	7.8	4,100	26.2	90000	NORMAL	NORMAL	N	3	2.492	43.26	0.02	0.41							
17	6	F	7	YES	NO	NO	NO	NO	YES	11.5	6,300	36.5	98000	NORMAL	NORMAL	N	7	0.076	1.35	1.032	21.58							
18	10	M	5	YES	YES	NO	NO	YES	YES	17.4	8,300	49.6	14000	NORMAL	NORMAL	N	5	0.273	4.73	0.032	0.66							
19	10	M	7	YES	YES	NO	NO	NO	YES	15.9	7,200	49	52000	NORMAL	BLPF	N	7	0.112	1.94	1.012	21.17							
20	11	F	4	YES	YES	NO	NO	YES	YES	12.1	3,700	39.9	1.1	NORMAL	NORMAL	N	4	0.061	1.05	0.04	0.83							
21	9	M	5	YES	YES	NO	NO	YES	YES	12.2	7700	38,3	44000	NORMAL	NORMAL	N	5	0.07	1.21	0.18	3.76	0.06	2.429	16	1.167	24.36	0.855	34.615
22	12	M	4	NO	YES	YES	NO	YES	YES	13.1	5000	39.9	33000	NORMAL	ASCITIS	N	4	2.35	40.79	2.4	50.104	2.36	95.55	16	2.428	50.69	2.414	97.73
23	10	M	4	NO	YES	NO	NO	YES	YES	12.5	3400	39	60000	NORMAL	PF/AS/GB	N	4	0.07	1.21	2.42	50.52	2.4	97.17	18	0.504	10.52	2.365	95.75
24	12	F	7	YES	YES	NO	NO	NO	YES	12	5300	39	98000	NORMAL	NORMAL	N	7	0.669	1.19	0.277	5.78	0.202	8.178	18	0.243	5.07	0.277	11.21
25	10	M	5	NO	YES	NO	NO	NO	YES	12.8	3800	40.2	1.2	NORMAL	NORMAL	ELEVATED	5	0.065	1.12	1.263	26.37	0.486	19.68	19	0.46	9.6	0.385	15.58
26	6	M	6	YES	YES	NO	NO	YES	YES	12.2	2900	36.7	84000	NORMAL	GB	HIGH	7	0.137	2.37	0.51	10.65	0.814	32.96	19	2.167	45.24	2.412	97.65
27	7	F	4	NO	NO	NO	NO	YES	YES	18.1	8200	55.9	28000	NORMAL	NORMAL	HIGH ENZ	6	0.048	0.83	2.43	50.73	2.419	97.94	18	2.423	50.58	2.425	98.18
28	8	M	2	NO	YES	NO	NO	NO	NO	11.6	13,400	38.1	47000	NORMAL	PF	N	2	0.057	0.98	2.48	51.77	2.46	99.19	15	2.366	49.39	2.499	101.1
29	4	M	7	NO	NO	NO	NO	YES	NO	10.2	9100	37.5	86000	NORMAL	PF,GB,	N	7	2.528	43.88	2.557	53.38	2.489	100.77	18	2.495	52.09	2.426	98.21
30	12	F	2	YES	YES	NO	NO	NO	NO	13.1	10,100	41.5	98000	NORMAL	NORMAL	N	2	0.056	0.97	2.055	42.9	0.237	9.59	13	0.27	5.64	0.279	11.29
31	6	F	8	YES	YES	NO	NO	NO	YES	12.8	6500	36	1.62	NORMAL	NORMAL	N	6	0.115	1.99	0.285	5.94	0.113	4.57	21	0.381	7.95	0.811	32.83
32	3	M	5	NO	YES	NO	NO	NO	NO	10.3	2,700	34.6	1.4	NORMAL	NORMAL	N	5	2.446	42.46	2.446	51.06	0.046	1.82	16	2.369	49.46	0.871	35.26
33	12	M	5	YES	NO	NO	NO	YES	NO	13.7	2700	43.2	96000	NORMAL	NORMAL	N	5	0.042	0.78	0.146	3.05	0.164	6.64	16	0.224	4.68	0.407	16.48
34	6	M	7	NO	YES	NO	YES	NO	YES	8.8	17,700	38	23000	NORMAL	HEPA	N	7	0.061	1.05	0.753	15.72	1.542	62.43	17	1.411	29.46	1.763	71.38
35	5	M	7	YES	YES	NO	NO	YES	YES	13.2	8200	39.4	68000	NORMAL	PF,A	N	5	2.173	37.72	2.441	50.96	0.795	32.19	16	2.432	50.77	2.353	95.26
36	4	M	5	NO	NO	YES	NO	NO	YES	11.2	3,800	35	92000	NORMAL	HEPA	N	5	0.056	0.97	2.502	52.23	0.526	21.29	15	2.502	52.23	0.336	13.68
37	2	F	4	NO	NO	NO	NO	NO	NO	10.7	13,600	34.1	70000	NORMAL	NORMAL	N	4	2.554	44.34	2.566	53.57	0.091	3.68	18	2.447	51.08	0.496	20.08
38	9	M	7	NO	YES	NO	NO	NO	YES	14.9	5,200	45.5	68000	NORMAL	HEPA	N	7	2.405	41.75	2.424	50.6	0.089	3.6	16	2.436	50.86	0.501	20.28
39	5	M	7	NO	YES	NO	NO	NO	NO	10	11,000		90000	NORMAL	NORMAL	N	7	0.113	1.96	0.373	7.79	0.026	1.01	22	0.224	4.68	0.33	1.33
40	2	F	5	NO	YES	NO	NO	YES	NO	12.3	7,000	38.3	49000	NORMAL	NORMAL	N	5	0.141	2.44	2.436	50.86	1.193	48.29	22	1.826	38.16	1.521	61.58
41	5	F	3	NO	YES	NO	NO	YES	NO	12.2	6,500	39.4	96000	NORMAL	BLPF	N	3	0.16	2.77	0.637	13.29	0.206	8.34	16	1.327	27.7	0.029	1.17
42	4	F	6	NO	YES	NO	NO	YES	NO	10.7	6,200	35	81000	NORMAL	HEPAT	N	4	2.485	43.1	2.3	48.01	0.147	5.95	16	2.471	51.59	1.337	54.12
43	8	F	3	NO	YES	NO	NO	NO	YES	11	5,400	34.4	25000	NORMAL	NORMAL	N	3	0.058	1	2.134	44.55	2.401	97.2	17	1.135	23.69	2.271	91.94
44	3	M	7	NO	YES	NO	NO	NO	NO	9.5	12,400	30	58000	NORMAL	PF	N	7	0.06	1.04	2.498	52.15	2.469	99.54	18	0.34	7.09	0.169	6.84
45	9	F	5	NO	YES	NO	YES	YES	NO	12.2	5,800	38	85000	NORMAL	NORMAL	N	5	0.055	0.95	1.011	21.1	0.315	12.75	18	0.386	8.05	0.153	6.19
46	8	M	7	NO	YES	NO	NO	YES	YES	15..7	11,800	47.7	18000	PF	PF,AS	ELEVATED	7	2.332	40.48	2.346	47.88	1.497	60.61	18	1.426	29.77	2.55	91.29
47	12	F	5	YES	YES	NO	NO	NO	NO	13	34,000	39	72000	NORMAL	NORMAL	N	5	0.076	1.31	0.209	4.36	0.783	31.7	18	0.31	6.47	0.719	29.1
48	8	M	1	YES	YES	NO	NO	NO	NO	12	8,300	37.3	1.2	NORMAL	NORMAL	N	3	2.457	42.6	1.293	26.99	0.146	5.91	18	2.54	53.02	0.308	12.47
49	8	M	7	YES	YES	NO	NO	YES	NO	9.6	4,600	35.7	71000	NORMAL	NORMAL	N	7	0.058	0.97	0.371	7.74	0.416	16.84	15	0.232	4.84	0.022	0.89
50	7	F	2	YES	YES	NO	YES	NO	NO	9.5	4,400	37	43000	NORMAL	NORMAL	N	2	0.057	0.98	0.267	5.57	0.051	2.06	17	2.441	50.96	2.382	96.44
51	11	F	9	YES	NO	NO	NO	YES	YES	9.9	4,600	31	2.01	NORMAL	LIV,SPLE	N	6	0.048	0.83	0.332	6.93	0.067	2.71	17	1.849	38.6	0.276	11.17
52	3	M	5	NO	YES	NO	NO	NO	YES	10.1	5,400	31.8	47000	NORMAL	PF	ELEVATED	5	0.144	2.5	2.482	51.82	2.497	101.9	19	2.4	50.1	2.493	100.93
53	5	M	5	NO	YES	NO	NO	YES	YES	12.9	18,000	43	55000	NORMAL	NORMAL	N	5	0.058	1	2.519	52.59	2.606	105.51	18	2.364	49.35	2.344	94.89
54	12	M	4	YES	NO	NO	NO	YES	NO	14	8,200	49	44000	NORMAL	NORMAL	N	6	0.046	0.79	2.363	49.33	2.364	95.71	18	2.42	50.52	2.415	97.77
55	7	F	6	NO	YES	NO	NO	NO	YES	12.2	5,000	39	78000	NORMAL	NORMAL	N	6	0.042	0	0.382	7.97	1.073	43.44	12	0.196	4.05	0.066	2.67
56	2	F	5	NO	YES	NO	NO	YES	YES	10.2	6,200	40.2	68000	NORMAL	NORMAL	N	5	0.078	1.35	0.244	5.09	0.045	1.821	16	0.542	11.31	0.594	24.04
57	7	F	7	YES	YES	NO	NO	YES	NO	13	5,000	39.9	46000	NORMAL	GB	HIGH	7	0.085	1.47	2.524	52.69	2.515	101.82	15	2.407	51.21	0.341	13.8
58	6	M	6	YES	NO	NO	NO	YES	NO	11	4,600	38	90000	NORMAL	PF	N	6	0.362	6.28	2.306	48.14	2.44	98.78	21	2.423	51.55	2.416	97.81
59	7	M	7	YES	YES	NO	NO	NO	NO	13.2	5,100	41.7	88000	NORMAL	NORMAL	N	6	2.433	42.23	2.437	50.88	0.044	1.781	22	2.449	51.13	0.202	8.18
60	8	M	5	YES	YES	NO	NO	YES	NO	11.3	13,600	33.9	80000	NORMAL	NORMAL	ELEVATED	5	2.337	40.57	1.201	25.07	0.119	4.817	12	2.519	52.59	0.196	7.93
61	10	M	3	YES	YES	NO	NO	YES	YES	13.1	58,000	39.9	20000	PF	PF,GB,LIV	N	4	2.556	44.4	2.576	53.78	0.138	5.587	17	0.316	6.59	0.126	5.1
62	5	M	5	NO	YES	NO	NO	NO	NO	14.5	9,300	36	46000	NORMAL	PF	N	5	0.04	0.69	2.406	50.23	2.376	96.19	17	2.364	49.35	2.385	96.56
63	6	F	7	NO	YES	NO	YES	YES	YES	15	9,200	45.3	29000	NORMAL	RF,AS	N	7	2.307	40.05	2.472	51.6	2.262	91.58	20	2.456	51.27	2.087	84.49
64	3	M	6	YES	NO	NO	NO	YES	NO	9.7	10,900	33.1	73000	RF	RF,AS	N	6	2.408	41.8	2.463	51.42	0.056	2.27	17	2.534	52.9	0.54	21.86
65	9	F	4	YES	YES	NO	NO	NO	YES	14.5	4,500	46.2	21000	NORMAL	RF	N	4	0.035	0.6	2.466	51.48	2.476	100.24	16	2.067	43.15	2.349	95.1

FIG – 1 DENGUE VIRAL GENOME

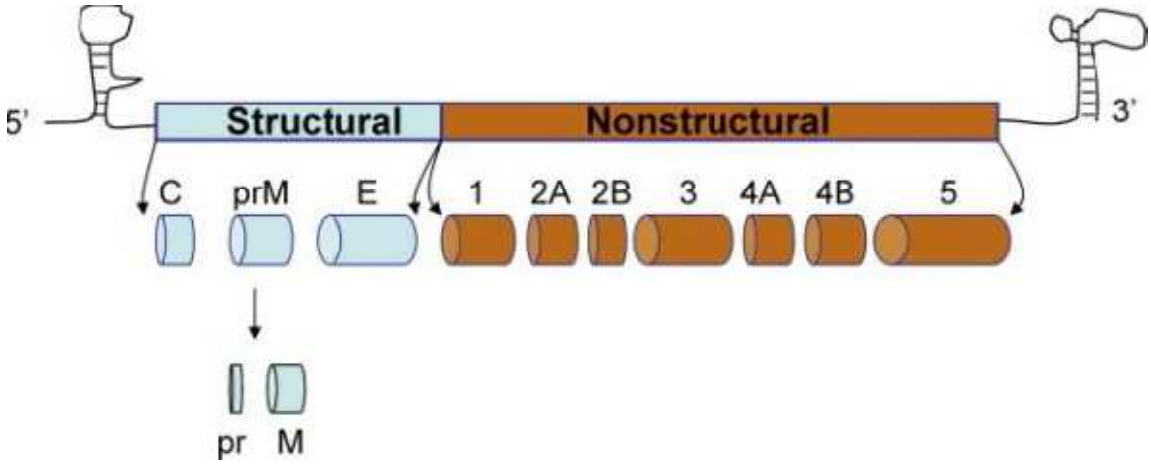
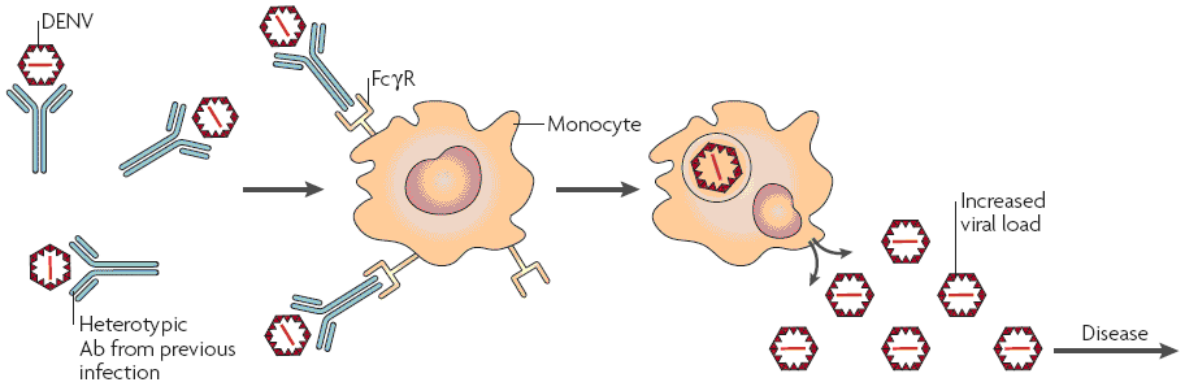
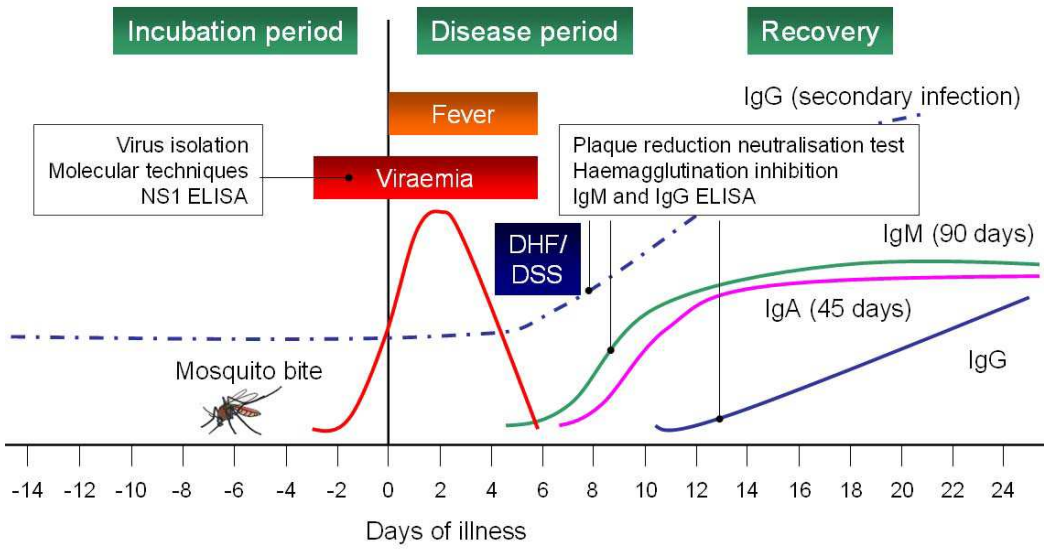


FIG 2 ANTIBODY DEPENDANT ENHANCEMENT-MECHANISMS



TIME COURSE OF CLINICAL ILLNESS OF DENGUE WITH DIAGNOSTIC MARKERS



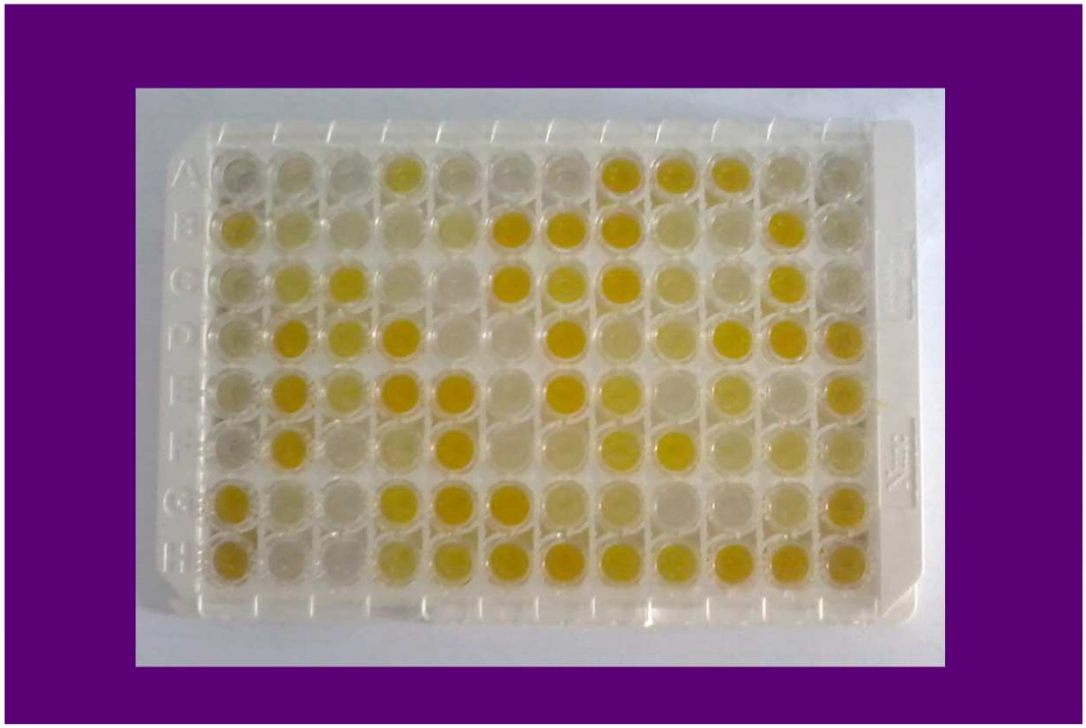
Procedure

Panbio Dengue ELIAS Kits
NS1, IgM, IgG

Automatic micro plate washing instrument

ELISA reader

ELISA



Panbio Dengue ELIAS Kits
NS1, IgM, IgG

Procedure

Automatic micro plate washing instrument

BIBLIOGRAPHY

1. World Health Organization. Comprehensive Guidelines for Prevention and Control of Dengue and DHF, 2011 WHO, Region of South-East Asia.
2. Maria Guzman,Scott Halstead. Effect of age on outcome of secondary dengue 2 infections. Intl Journal of Infectious Diseases. 2002;6(2):118–24.
3. Knipe D, Roizman B. Fields Virology. Fifth. Lippincot Williams and wilkins; 2005.
4. Ramakrishnan SP, Mukherjee RN. The epidemic of acute haemorrhagic fever, Calcutta, 1963: Indian J Med Res. 1964 Jul;52:633–50.
5. Bharaj P, Guleria R, et al. Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. Virol J. 2008 Jan 9;5:1–1.
6. S.C. Tewari, A. Gajanana. Dengue vector prevalence and virus infection in a rural area in south India. Tropical Medicine & International Health. 2004 Apr 13;9(4):499–507
7. Gubler DJ, Clark GG. Dengue/DHF: the emergence of a global health problem. Emerg Infect Dis. 1995;1(2):55–7.

8. Capeding RZ, Yoon I-K, et al. The Incidence, Characteristics, and Presentation of Dengue Virus Infections during Infancy. Am J Trop Med Hyg. 2010 Feb;82(2):330–6.
9. McBride WJ, Ohmann H. Dengue viral infections;pathogenesis and epidemiology. Microbes Infect. 2000;2:1041–50.
10. Rebeca Rico-Hesse,Améliada Rosa. Origins of Dengue Type 2 Viruses Associated with Increased Pathogenicity in the Americas. Virology Journal. 1997 Apr 14;230(2):244–51.
11. Vaughn DW, Suntayakorn S, et al. Dengue Viremia Titer, Antibody Response Pattern, and Virus Serotype Correlation with Disease Severity. Journal of Infectious Diseases. 2000 Jan 1;181(1):2–9.
12. Guy B, Saville M, Lang 205. J. preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* 2011; *29* : 7229-41.
13. Sabin, A. B. 1952. Research on dengue during World War II. *Am. J.Trop Med Hyg*, 1: 30–50.
14. Graham RR, Sutaryo, Halstead SB.(1999) A prospective seroepidemiologic study on dengue in children four to nine years I. studies in 1995-1996.*Am J Trop Med Hyg*.; 61(3):412-9.
15. Thai K,de Vries P (2005);Seroprevalence of dengue antibodies, annual incidence and risk factors among children. *Trop Med Int Health.* 10(4): 379-386

16. Balmaseda A, Tellez Y, (2006) High seroprevalence of antibodies against dengue virus in a prospective study of schoolchildren.*Trop Med Int Health*.; 11(6): 935-942

17. Comach G, Camacho D, (2008)Dengue Virus Infections in a Cohort of Schoolchildren . Vector Borne Zoonotic Dis

18. Ukey, Akulwar, S.L., 2010. Study of seroprevalence of dengue fever in Central India.

19. Neeraja M, Subbalakshmi (2006) Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. *Indian Journal of Medical Microbiology* **24**, 280–282.

20. Gupta N, Chaturvedi UC. Dengue in India. Indian J Med Res. 2012 Sep;136(3):373–90.

21. Gunasekaran, Sheriff. Dengue disease status in Chennai (2006-2008): A retrospective analysis

22. John Victor, Padmanaban, P., 2007. Laboratory-based dengue fever surveillance in Tamil Nadu, India. *Ind. J. Med. Res,* **126:** 112-115.

23. Bhaskar, M. Senthil Kumar, N. 2010. Dengue haemorrhagic fever among adults - An observational study in Chennai, South India.

24. Paramasivan, Tyagi, B.K., Dengue fever caused by dengue virus serotype – 3 in a rural area of Madurai district, Tamilnadu.

25. Gupta E, Broor S. The changing epidemiology of dengue in Delhi, India. *Virol J* 2006 ; *3* : 92-6.

26. Barbazan P, Gonzalez JP ,DHF epidemiology in Thailand: description and forecasting of epidemics. Microbes Infect. 2002;4:699–705
27. Ratho, Varma, S., 2006. Dengue fever /DHF in Chandigarh (North India). *Dengue Bulletin*, **20.**
28. Singh J, Bhardwarj et al. (2000) Silent spread of dengue and DHF to Coimbatore and Erode districts in Tamil Nadu, India 1998: Epidemiology and Infection 125, 195–200.
29. Arunachalam N, Thenmozhi V, et al. (2004) Studies on dengue in rural areas of Kurnool , India. J Am Mosq Control Assoc 20: 87-90.
30. Nisalak A, Endy TP, et al. Serotype-specific dengue virus circulation and dengue disease in Bangkok.Am J Trop Med Hyg 2003;68:191–202
31. Guzman, M.G. and Kouri, G., 2002. Dengue: an update. *Lancet. Infect. Dis*, **2:** 33–42.
32. Wichmann, Ole, et al. "Risk factors and clinical features associated with severe dengue infection in adults and children " *Tropical Medicine & International Health* 9.9 (2004): 1022-1029.
33. Kabra SK, Pandey RM, et al. DHF in children in the 1996 Delhi epidemic. Trans R Soc Trop Med Hyg 1999;93:294–8.
34. Narayanan M, Aravind MA, et al. Dengue fever epidemic in Chennai—a study of clinical profile and outcome. Indian Pediatr2002;39: 1027–33.

35. Gomber S, Gupta P, *et al*. Hematological observations as diagnostic markers in dengue hemorrhagic fever . Indian Pediatr 2001; 38: 47.
36. Aggarwal A, Dutta AK. An epidemic of DHF and DSS in children in Delhi. *Indian Pediatr* 1998; *35* : 727-32.
37. Thisyakorn U, Nimmannitya S. Nutritional status of children with DHF. Clin Infect Dis 1993;16:295–7.
38. Vinod H Ratgeri, P N Yargolakar. Clinical profile and outcome of dengue fever cases Indian J Pediatr (2005) 72(8) 705-706.
39. Kabilan, L., et al. "Dengue disease spectrum among infants." *Journal of clinical microbiology* 41.8 (2003): 3919-3921.
40. Alcon S, Talarmin A,ELISA specific to dengue virus type-1 NS1 reveals circulation of the antigen in the blood during acute phase in patients experiencing primary or secondary infections. *J Clin Microbiol* 2002; 40: 376-81.
41. Xu H,Hao W, et al. Serotype 1-Specific Monoclonal Antibody-Based Antigen Capture Immunoassay for Detection of Circulating NS1:. Journal of Clinical Microbiology. 2006 Aug;44(8):2872–8.
42. Dussart P, Lagathu G, *et al*. Evaluation of an enzyme immunoassay for detection of dengue v NS1 antigen in human serum. *Clin Vaccine Immunol* 2006; 13: 1185-9.
43. Singh, Ratho, 2010. Nonstructural protein NS1: giving a new structure to dengue diagnosis. *J. Clin. Microbiol,* **48(12):** 4688-4689.

44. Bessof K, Hunsperger E. Comparison of two commercially available (DENV) NS1 capture ELIZA using a single clinical sample for diagnosis of acute DENV infection. *Clin Vaccine Immunol* 2008; 15: 1513-8.

45. Shrivastava A, Lakshmana Rao PV. Evaluation of a commercial Dengue NS1 ELISA for early diagnosis of dengue infection. Indian J Med Microbiol 2011;29:51-5.

46. Pok K, Sng J, : Evaluation of NS 1 Antigen Assays for the Diagnosis and Surveillance of Dengue . Vector Borne Zoonotic Dis 2010.

47. Duong V, Cgroeung N, et al. Variation of NS1 antigen measured by commercial ELISA kit in various forms of dengue infections.BMC Proceedings. 2011;5:47

48. Chua KB, Wahab AH et al. A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation

49. Thomas L, Césaire R. Influence of the dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a dengue-2 and dengue-4 co-epidemic.Am J Trop Med Hyg. 2008:990–998.

50. Bisordi I,Maeda AY, *et al.* Evaluation of dengue NS1 antigen detection for diagnosis in public health laboratories, 2009. Rev Inst Med Trop Sao Paulo. 2011;53:315-20.

51. Pan American Health Organization. (1994) *Dengue and DHF in the Anericas: Guidelines for Prevention and Control.* Scientific Publication Nº. 548.

52. Sang,Devine. "Evaluation of a commercial capture ELISA for detection of Ig M and G antibodies produced during dengue infection." *Clinical and diagnostic laboratory immunology* 5.1 (1998): 7-10.

53. Innis BL. Nimmannitya S, et al. An Elisa to characterise dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 1989; 40: 418-427.

54. Prince, Mary -Nixon. "Utility of IgM/IgG ratio and IgG avidity for distinguishing primary and secondary dengue virus infections." *Clinical and Vaccine Immunology* 18.11 (2011): 1951-1956.

55. Hu, Xiaoyan Che. "Kinetics of non-structural protein 1, IgM and IgG antibodies in dengue type 1 primary infection." *Virol J* 8 (2011): 47.

56. Kassim, Saat. "Use of dengue NS1 antigen for early diagnosis of dengue virus infection." *The Southeast Asian journal of tropical medicine and public health* 42, no. 3 (2011): 562.

57. Blacksell, Thongpaseuth, S. et al Evaluation of the Panbio dengue virus NS1 antigen detection and immunoglobulin M antibody ELISA for the diagnosis of acute dengue infections. *Diagnostic microbiology and infectious disease*, 2008;*60*(1), 43-49

58. Gowri Sankar, John Vennison. "Evaluation and use of NS1 IgM antibody detection for acute dengue virus diagnosis: " Clinical Microbiology and Infection (2012).

59. Sathish,G. Sridharan. "Comparison of IgM capture ELISA with a commercial rapid immunochromatographic card test & IgM microwell ELISA for the detection of antibodies to dengue viruses." The Indian journal of medical research 115 (2002): 31.

60. Kuno G. (1997) Factor Influencing the transmission of dengue viruses. *Dengue and DHF*. Eds.CAB International, New York;1997:61-80.

61. Schilling, Schmitz, H (2004). Laboratory diagnosis of primary and secondary dengue infection. *Journal of Clinical Virology*; 31,3:179-184.

62. Chakravarti,Sonia Malik. "Detection of dengue infection by combining the use of an NS1 antigen based assay with antibody detection." Southeast Asian Journal of Tropical Medicine, no. 2 (2011): 297.

63. Vaughn DW, Kneen R, *et al.* Rapid serologic diagnosis of dengue virus infection using a commercial capture ELISA that distinguishes primary and secondary infections. Am J Trop Med Hyg 1999; 60: 639-698.

64. Miagostovich,V. Vorndam. "Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis." Journal of clinical virology 14, no. 3 (1999): 183-189.

65. Inoue S, Dimaano EM, et al. Evaluation of a dengue IgG indirect ELISA and a Japanese encephalitis IgG indirect ELISA for diagnosis of secondary dengue virus infection. Vector Borne Zoonotic Dis 2010; 10:143-150.

66. Vazquez S, Guzman MG. Evaluation of immunoglobulin M and G capture ELISA Panbio kits for diagnostic dengue infections. J Clin Virol 2007; 39:194-198

67. Hunsperger EA,Buchy P et al.Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Emerging Infect. Dis. 15(3),436–440 (2009)

68. Falconar, Claudia ME . "Altered ELISA immunoglobulin M (IgM)/IgG optical density ratios can correctly classify all primary or secondary dengue virus infections " Clinical and vaccine immunology 13, no. 9 (2006): 1044-1051.

69. Kumarasamy V,Wahab AHA, *et al*. Evaluating the sensitivity of a commercial dengue NS1 antigen capture ELISA for early diagnosis of acute dengue virus infection. *Singapore Med J* 2007; 48: 669-72

70. SD Sekaran, B. M. Kanthesh. "Sensitivity of dengue virus NS-1 detection in primary and secondary infections." *African Journal of Microbiology Research* 3, no. 3 (2009): 105-110.

71. Young , Halloran W. An antigen capture ELISA reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *J Clin Microbiol* 2000; 38: 1053-7

72. Koraka P,Falconar A, *et al*. Detection of immune-complex-dissociated NS1 antigen in patients with acute dengue virus infections. *J Clin Microbiol* 1993; 41: 4154-9

73. Lapphra, K., V. Thamlikitkul. 2008. Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness. Diagn. Microbiol. Infect. Dis.60387-391

74. Chandrakanta,AND R. Nagar. "Changing clinical manifestations of dengue infection in North India." Dengue Bulletin 32 (2008): 118-125.

75. Mittal, Patil. "Clinicohematological profile and platelet trends in children with dengue " *Indian journal of pediatrics* (2012): 1-5.

76. Kumar, Samarasinghe. "Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, Udupi district." *Indian journal of community medicine:* 35, no. 3 (2010): 386.

77. Khan, Hasan. "Dengue outbreak in Karachi, Pakistan, 2006: " Transactions of the Royal Society of Tropical Medicine and Hygiene (2007): 1114-1119.

78. Batra,Saha,Chaturvedi;outbreak of dengue infection in rural maharashtra;Indian j paediatrics;2007,74;794-5.

79. Balasubramanian S, Janakiraman L, Kumar SS, et al. A reappraisal of the criteria to diagnose plasma leakage in dengue hemorrhagic fever. Indian Ped 2006; 43: 334–339

80. Kulkarni , Shubhada RM et al. Association of platelet count and serological markers of dengue infection. Indian J Med Microbiol 2011; 29(4): 359-6

81. Ramirez , Comach G, *et al*. Evaluation of dengue NS1 antigen detection tests with acute sera from patients infected with dengue virus. *Diagn Microbiol Infect Dis* 2009; 65: 247-53.

82. Pancharoen C & Thisyakorn U (2001) Dengue virus infection during infancy. Transaction of the Royal Society of Tropical Medicine and Hygiene. 95, 307–308.

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ANNEXURE I- PROFORMA

DATA SHEET FOR COLLECTION OF SOCIO DEMOGRAPHIC, CLINICAL AND LABORATORY DATA

FOR P.G. DISSERTATION WORK ON

"SERODIAGNOSIS OF DENGUE IN PAEDIATRIC PATIENTS -

IMPORTANCE OF NS1 ANTIGEN"

Name		Age		Sex		Hospital No.	

Address Phone No.	

FEVER	
Duration	
Rigor	
Vomiting	
Retro orbital pain	

BACK PAIN	
MYALGIA	
ARTHRALGIA	

MACULOPAPULAR RASH	
started on	
spread	

Hemorrhagic manifestaions	
Petechiae	
Echymosis	
Bleeding gums	
GIT bleeding	
Haematuria	

Respiratory symptoms	
Sore throat	
Rhinitis	
Cough	
Symptoms of Hepatitis	
Jaundice	

Symptoms of CNS involvement	
Altered consciousness	
Focal neurological defecit	
Fits	

Symptoms of shock	
Hypothermia with sweating	
Restlessness / refusal of feeds	
Somnolence	

Previous episode	
Family H/O Dengue	
Past H/O hospitalization with platelet transfusion	

H/O mosquito bite	
Presence of stagnant water around the living area	

GENERAL EXAMINATION

Built		Petechiae		Weight	
Anaemia		Rash		Temperature	
Jaundice		Conjunctival congestion		Heart rate	
Cyanosis		Pedal edema		RR / SaO_2 %	
Lymphadenopathy		Tourniquet test		BP	

SYSTEMIC EXAMINATION:

CVS	
RS	
CNS	
Abdomen	

INVESTIGATIONS:

Hb.		RBS			
TC.		Sr.Protein			
DC.				Urea	
ESR		creatinine			
HCT %		Urine deposits			
PLATELETS					
LFT					
Bld culture		Typhoid		Leptospirosis	
Malaria		chickungunya		JE	
USG					
CXR					

SEROLOGY:

Tests	**I – sample**	**II – sample**
NS1		
IgM		
IgG		